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On Renal Injuries in Young Rats on Choline Deficiencies

By

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Marked hemorrhagic degeneration of the kidney has been reported in young rats kept on a choline deficient diet. (Griffith & Wade, 1939, 1940;[1),2)] György & Goldblatt, 1940;[3)] Christensen, 1941;[4)] Engel & Salmon, 1941[5)])

In experiments that we performed, renal lesions occurred in 5 of 9 weanling rats kept on a choline deficient diet. In studies of dietary fatty liver cirrhosis, we have observed hemorrhagic degeneration of the kidney in some male adult rats fed a choline deficient diet.

Experimental

Methods

Experiment I

Four young male rats weighing an average of 21.6 g. (15.7 to 24.2 g.) and five young female rats weighing an average of 20 g. (17.0 to 22.5 g.) were fed a choline deficient diet ad libitum. The composition of the diet is indicated in Table I.

As controls, five young rats (1 male and 4 female) having an average body weight of 26.6 g. (22.3 to 30.1 g.) were fed the same diet plus 20 mg. choline chloride per rat per day. All rats were killed after seven days of experimental diet.

Experiment II

In studies of dietary fatty liver cirrhosis, three adult male rats which were fed a choline deficient diet, died after 12, 100 and 131 days and showed renal changes.

TABLE I

Composition of Diet

	Young rats (16–30 g.)		Adult rats (69–210 g.)
	Choline deficient diet	Control	Choline deficient diet
Casein	18%	18%	18%
Sucrose	57 "	57 "	48 "
Lard	20 "	20 "	38 "
Osborne-Mendel's salt mixture	5 "	5 "	4 "
Cod liver oil	—	—	2 "
Choline chloride	—	20 mg	—
Vitamin (per rat per day)			
Thiamine	50 γ	50 γ	50 γ
Riboflavine	50 "	50 "	50 "
Pyridoxine	20 "	20 "	20 "
Calcium-pantothenate	100 "	100 "	100 "
Nicotinamide	500 "	500 "	500 " (after 12 weeks)
Cod liver oil (fortified)	1 drop/2 days	1 drop/2 days	—
Alpha-tocopherol	5 mg/week	5 mg/week	10 mg/week

TABLE

Experimental

		Rat No.	Sex	Experimental period	Body weight at start	final	Weight of kidney
Young rats	Choline deficient diet	101	M	7 days	24.0 g.	27.2 g.	522 mg.
		104	M	7 "	24.2 "	35.0 "	667 "
		108	M	7 "	22.6 "	28.2 "	714 "
		109	M	3 "	15.7 "	13.2 "	346 "
		102	F	7 "	17.0 "	22.2 "	433 "
		103	F	7 "	19.7 "	27.0 "	338 "
		105	F	7 "	20.1 "	31.5 "	723 "
		106	F	7 "	20.5 "	32.2 "	456 "
		107	F	7 "	22.5 "	23.9 "	319 "
	Control diet	111	M	7 "	27.2 "	43.9 "	434 "
		110	F	7 "	22.3 "	39.0 "	387 "
		112	F	7 "	25.3 "	37.9 "	371 "
		113	F	7 "	30.1 "	44.5 "	476 "
		114	F	7 "	28.0 "	41.4 "	400 "
Adult rats	Choline deficient diet	51	M	12 "	84.0 "	68.4 "	1700 "
		45	M	100 "	99.0 "	76.5 "	800 "
		7	M	131 "	100.0 "	85.5 "	1400 "

The composition of diet is indicated in Table I.

In all rats, histo-pathologic studies were carried out and liver fat and serum protein levels were determined.

Results

Experiment I

The average daily food intake was 3.04 g. in rats receiving the choline deficient diet snd 4.75 g. in the control group. The average increase of weight during the 7-day experimental diet period was 6.01 g. for the choline deficient diet group and 10.63 g. in the contorol group. The smallest male rat died after three days of diet, another male died on the seventh day and the remaining 7 rats were killed after seven days of diet.

Macroscopic renal changes were recognized clearly in all 4 male rats, but in only one of the 5 female rats. No changes were recognized in the kidneys of the 5 rats receiving the control diet.

Macroscopically, the kidneys which showed renal injuries were enlarged and dark red in colour, with white flecks giving them a finely mottled appearance. Expressed as the ratio of kidney weight to body weight, kidneys with microscopically recognizable renal injuries were 2.2 times heavier than contorols, and even the kidneys which had no recognizable renal injuries were 1.4 times heavier than the contorols.

II

Results

Weight of kidney / Weight of body	Liver fat (Wet %)	Partition of serum protein					Renal injuries
		Alubumin %		Globulin %			
		α_1	α_2	α	β	γ	
1.91%	9.4%						##
1.91 "	19.1 "	37.4	16.2	11.4	17.9	17.1	##
2.53 "	11.5 "	36.4	24.6	13.6	14.4	11.0	##
2.62 "	5.1 "						##
1.95 "	8.0 "						+
1.25 "	8.3 "	35.0	14.9	11.0	23.4	15.6	+
2.30 "	18.9 "						##
1.22 "	17.7 "	47.4	14.7	7.8	17.2	12.9	+
1.33 "	5.9 "						+
0.99 "	6.9 "	32.5	19.0	15.9	17.5	15.1	−
0.99 "	8.5 "	42.2	12.2	13.8	19.5	12.2	−
0.98 "	7.4 "	41.6	12.4	14.1	19.5	12.4	−
1.07 "	8.8 "	42.7	15.3	14.5	18.1	9.3	−
0.97 "	7.8 "	37.7	17.9	14.1	17.0	13.2	−
2.49 "	13.8 "						###
1.05 "	5.8 "						
1.40 "	35.8 "						

The livers of rats on the choline deficient diet were enlarged, the ratio of liver weight to body weight being 6.37% as compared to 5.19% for the contorols. Liver fat content was increased in rats eating a choline deficient diet, being 10.99% as compared with 7.88% for the contorols.

Partition of serum proteins by paper electrophoresis revealed no recognizable differences between rats fed a choline deficient diet and those fed the contorol diet. Thymus gland weight, expressed as percent of body weight, was 0.19% for rats on the choline deficient diet compared with 0.36% for contorols. Conversely, spleen weight, expressed as percent of body weight, was greater in rats fed a choline deficient diet (0.53% compared with 0.34% for contorols).

Histopathologic changes included remarkable degenerative necrosis of the tubular epithelium. Cloudy swelling was prominent, particularly near or around the convoluted tubules. The tubular lumen was indistinct or obliterated. (Fig. 1) In Sudan III stained sections, irregular granules of various sizes were observed in degenerating cells. In the more severely damaged kidneys, massive degeneration was observed extending from one tubule to adjacent tubules, often with hemorrhage. Hyaline and red blood cell casts were seen in the distal segments of the renal tubules and collecting systems (Fig. 2) Generally, the glomeruli were hyperamic and more severe changes were not present but in some areas of necrosis, hemorrhagic degeneration involved the glomeruli as well as other structures.

Fig. 1

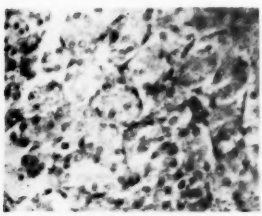

Fig. 2

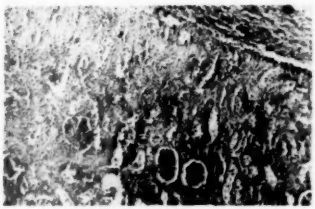

Fig. 1. Remarkable degenerative necrosis of the tubular epithelium. Hematoxylin and eosin staining. 540×.

Fig. 2. Massive degeneration extending from one tubule to adjacent tubules, often with hemorrhage, in severely damaged kidneys. Some staining. 180×.

Experiment II

Some of adult rats showed acute renal changes such as seen in experiment I and in the kidneys of some of rats, increase of connective tissues such as wedge-shaped, was recognized and in that tissues was observed

a relative healthy glomeruli or atrophic tubular lumens. Even if a fibrosis of high grade such as apparently infarction was not recognized, nest-form increase of connective tissues was often recognized. (Fig. 3) In

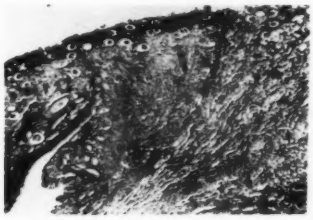

Fig. 3. Increases of connective tissues such as wedge-shaped and a relative healthy glomeruli or atrophic tubular lumens. Van Gieson's staining. 180×.

judging from these pathologic changes it is thought that the massive necrosis was gradually transported to the fibrosis with the regeneration of tubular epithelium, finally, such as wedge-shaped changed for cicatrization.

Conclusions

The renal changes occurring in these experiments were similar to the acute hemorrhagic degeneration reported by Engel & Salmon (1941)[5]; Griffith & Wade (1939)[1]; Christensen (1942)[6] and György & Goldblatt (1940)[3]. Renal damage occurred in 5 of rats; an incidence of 55.6%. This can be compared with an incidence of 50% after six to ten days (Engel & Salmon)[5]; 100% after five to seven days (Dessan)[7] and 98% after ten days (Griffith & Wade)[1]. According to Griffith, male rats 20–30 days of age are susceptible to the renal damaging effects of a choline deficient diet, but male rats 33 days of age or older and all female rats are relatively resistant. Though the ages of rats in our experiments were unknown, it was thought to be about 30 days. All male rats developed renal changes, but recognizable changes were seen in only one of five female rats.

The lack of change in serum proteins was not unexpected in view of the short duration of the studies.

The thymus of rats kept on a choline deficient diet weighed less than that of controls, whereas the spleen weighed more than that of the controls.

These changes were the same as those described in the above reports.

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Urinary Output of Aminoacid Nitrogen of Children with Nutritional Dystrophy and the Effect of Riboflavine or Nicotinamide upon It

Studies on the Nutrition of Children in Hirosaki Area

(46th Report)

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In the children with nutritional dystrophy[1], dysproteinemia[2] and an abnormal increase in the urinary excretion of the methionine ingested orally[3] were found, so a disturbance in aminoacid metabolism of these children was suspected. In the present paper, urinary output of aminoacid nitrogen and an effect of riboflavine or nicotinamide upon it will be dealt with.

Experimental

Methods and Material

The aminoacid nitrogen in the urine was estimated with Copper method[4], creatinine with Jaffe's method[5] and total nitrogen with micro-Kjeldahl. The 24 hour-urine specimens were used throughout this investigation. The children subjected to this experiment were all the boys at the age of 7 to 8 years and they were divided into 2 groups (Groups I and II). Group I included 15 boys who, though apparently healthy, showed ariboflavinotic signs as part manifestations of nutritional dystrophy[1]. Group II included 15 boys who were healthy and free from ariboflavinosis.

The effect of riboflavine or nicotinamide upon urinary aminoacid nitrogen was examined in the following way: all the 15 boys of Group I were divided into 3 Subgroups (Subgroups B_2, N and K), each of which included 5 boys with nutritional dystrophy. Subgroup B_2 was given 5 mg. of riboflavine and Subgroup N was given 50 mg. of nicotinamide. Subgroup K—with no medication—was the control group to each of the other subgroups. Riboflavine or nicotinamide was given orally every day

for 72 days. The aminoacid nitrogen, creatinine and total nitrogen in the 24 hour urine were estimated before (20/IV, 1955), on the 8th (28/IV), the 19th (9/V), the 42nd (1/VI) and the 72nd day (1/VII) of the administration of riboflavine or nicotinamide.

TABLE I

Aminoacid Nitrogen, Creatinine Nitrogen and Total Nitrogen in the 24 Hour Urine of Children with with Nutritional Dystrophy (Group I)

Before Administration of Riboflavine or Nicotinamide

Subgroup	No. of case	Name and age (years and months)	Amount of the 24 hour urine cc.	Aminoacid N		Creatinine -N mg.%	Aminoacid-N (mg. %) / Creatinine-N (mg. %)	Total nitrogen mg.	Percentage of amino-acid-N (mg.) to total-N (mg.)
				mg. %	mg.				
	1	T.S. 8.0.	1200	20.0	240.0	15.6	1.28	7770	3.0
	2	I.K. 7.10.	1275	14.0	178.5	10.0	1.40	5190	3.4
B_2	3	Z.Y. 7.1.	1390	15.5	215.4	6.3	2.46	3419	6.3
	4	N.S. 7.10.	980	17.0	166.4	14.1	1.21	5576	2.9
	5	K.M. 7.3.	590	23.5	138.6	11.5	2.04	3439	4.0
	6	N.M. 7.2.	975	7.0	68.2	7.0	1.00	2340	2.9
	7	K.K. 8.0.	1310	11.0	144.0	7.8	1.41	4010	3.5
N	8	T.T. 7.6.	1350	15.5	209.2	10.0	1.55	4500	4.6
	9	K.S. 7.1.	1470	14.0	205.8	8.2	1.71	4730	4.3
	10	T.K. 7.5.	1450	14.0	203.0	12.2	1.15	7740	2.6
	11	K.Y. 8.5.	1440	12.5	180.0	6.3	1.98	3310	5.4
	12	K.A. 7.7.	615	23.5	144.4	14.1	1.67	4940	2.9
K	13	A.K. 8.1.	525	23.5	123.2	20.0	1.17	3200	3.8
	14	H.M. 7.2.	1150	17.0	195.4	11.5	1.47	5680	3.4
	15	N.Y. 7.5.	1425	12.5	178.0	8.2	1.52	4750	3.7
Average for Group I			1143 ±142	16.0 ±2.6	172.6 ±18.5	10.9 ±1.9	1.53 ±0.23	4706 ±860	3.7 ±0.6

Results and Discussion

Urinary output of aminoacid nitrogen in the children with or without nutritional dystrophy

The results were given in Tables I and II (Cf. Tables I and II). The average values for aminoacid nitrogen in the 24 hour-urine were 172.6 ±18.5 mg. and 91.8±21.1 mg. in Groups I and II respectively, showing a significantly higher value in the former than in the latter. According

TABLE II

Aminoacid Nitrogen, Creatinine Nitrogen and Total Nitrogen in the 24 Hour Urine of Children without Nutritional Dystrophy (Group II)

No. of case	Name and age (years and months)	Amount of the 24 hour urine cc.	Aminoacid-N		Creatinine -N mg.%	Aminoacid-N (mg.%) / Creatinine-N (mg.%)	Total nitrogen mg.	Percentage of amino-acid-N to total nitrogen
			mg.%	mg.				
1	Y.T. 8.3.	685	8.2	56·4	13.0	0.62	3041	1.8
2	N.Y. 8.3.	610	10.0	61.0	8.5	1.17	3336	1.8
3	M.T. 7.7.	1280	7.0	89.6	7.8	0.89	3730	2.4
4	T.H. 7.11.	870	17.0	147.8	17.8	0.95	6385	2.3
5	N.K. 7.10.	640	18.5	118.4	17.8	1.03	5145	2.3
6	0.	650	14.0	91.0	14.1	0.99	3300	2.7
7	H.O. 7.9.	960	13.2	127.2	13.0	1.01	6910	1.8
8	K.T. 7.9.	885	7.0	61.8	11.1	0.63	4820	1.2
9	I.T. 8.2.	630	10.0	63.0	14.7	0.68	3960	1.5
10	T.K. 7.7.	440	20.0	88.0	24.3	0.82	4580	1.9
11	0.	890	20.0	178.0	18.2	1.09	7030	2.5
12	M.U. 8.1.	380	17.0	64.6	23.4	0.72	3980	1.6
13	M.K. 8.1.	380	26.6	101.0	21.3	1.24	4180	2.4
14	O.M. 7.9.	560	17.0	95.2	19.3	0.87	4380	2.1
15	I.A. 7.7.	4ε0	8.2	35.4	11.1	0.73	2110	1.6
Average		686 ±139	14.2 ±3.3	91.8 ±21.1	15.7 ±2.8	0.90 ±0.01	4459 ±785	1.9 ±1.4

TABLE III

The Amount of the 24 Hour Urine of Subgroups B, N and K

Subgroup	No. of case*	The amount of the 24 hour urine cc.				
		Before	Days of administration of riboflavine or of nicotinamide			
			8 days	19 days	42 days	72 days
Subgroup B_2	1	1200	625	640	781	
	2	1275	1415	1265	765	1240
	3	1390	1260	1115	1046	1500
	4	980	980	990	489	590
	5	590	900	400	780	350
	Average	1087	1036	882	772	920
Subgroup N	6	975	1030	1460	1383	1550
	7	1310	830	1440	1102	1520
	8	1350	1110	1215	1068	790
	9	1470	1785	1365	973	1270
	10	1450	1025	835	753	670
	Average	1311	1156	1225	1055	1160
Subgroup K	11	1440	1150	1615	1023	1860
	12	615	370	750	517	490
	13	525	815	770	708	1050
	14	1150	1545	965	630	1070
	15	1425	1630	1325	1282	1590
	Average	1031	1102	1085	832	1212

* No. of case in Table I and Tables III-VII shows the same case, so that No. 1 in Tables III-VII is No. 1 in Table I.

to Childs[6], in the normal children of the ages from 7 to 8 years, about 60 mg. of aminoacid nitrogen were excreted in the 24 hour-urine. This value for the normal children was considerably lower than that obtained from our own normal children (Group II), but this discrepancy will be explained by the difference between the methods adopted, because, according to Thompson *et al.*[7], the copper method, with which my dates were obtained, used to give about twice as high values as Van Slyke's method which was used in Childs's[6] investigation.

The ratios of the concentration of aminoacid nitrogen to that of creatinine in the 24 hour urine were 1.53 ± 0.23 and 0.90 ± 0.01 in Groups I and II respectively, and the former was significantly higher than the latter (Cf. Tables I and II).

The percentages of aminoacid nitrogen to total nitrogen in the 24

TABLE IV

The Aminoacid Nitrogen in the 24 Hour Urine of Subgroups B_2, N and K

Subgroup	No. of case*	Aminoacid nitrogen (mg.) in the 24 hour urine				
		Before	Days of administration of riboflavine or of nicotinamide			
			8 days	19 days	42 days	72 days
Subgroup B_2	1	240.5	156.2	80.0	78.0	
	2	178.5	198.0	19.4	63.0	18.6
	3	215.4	277.2	167.2	198.6	105.0
	4	166.6	166.6	116.2	83.0	82.6
	5	138.6	153.0	50.0	97.0	38.5
	Average	187.8	190.2	96.5	103.9	61.1
Subgroup N	6	68.2	113.2	40.0	133.2	23.2
	7	144.0	128.6	39.4	137.6	22.8
	8	209.2	188.6	85.0	197.4	55.3
	9	205.8	198.4	136.0	165.4	38.1
	10	203.0	174.2	104.2	150.6	83.7
	Average	166.0	160.6	80.9	157.8	44.6
Subgroup K	11	180.0	178.2	133.0	143.2	55.8
	12	144.4	111.0	138.6	129.2	75.9
	13	123.2	138.4	115.4	166.2	115.5
	14	195.4	262.6	144.6	97.6	64.2
	15	178.0	163.0	132.4	141.0	135.1
	Average	164.2	170.6	132.8	135.4	89.3

* See Footnote to Table III.

hour-urine were 3.7 ± 0.6 and 1.9 ± 0.4 in Groups I and II respectively, being significantly higher in the former.

An increase in the urinary aminoacid nitrogn was reported in rickets[8], scurvy[8], cirrhosis of the liver[9], deficiency in riboflavine[10] or tryptophan[10], or during an administration of ACTH or cortison[11),12]. According to Jonixs *et al.*[8], an increase in urinary output of aminoacid nitrogen resulted either when renal threshold was decreased or when plasma level of aminoacid nitrogen was elevated. In the children with nutritional dystrophy, a slight elevation of aminoacid nitrogen in plasma was found by the author[18], and an increased output of the methionine ingested orally was observed by Morinaga[3] in our Laboratory. So an increased output of aminoacid nitrogen in the urine of children with nutritional dystrophy will be ascribed partly to an aminoacidemia, even

TABLE V

The Total Nitrogen in the 24 Hour Urine of Subgroups B_2, N and K

Subgroup	No. of case*	Total nitrogen (mg.) in the 24 hour urine				
		Before	Days of administration of riboflavine or of nicotinamide			
			8 days	19 days	42 days	72 days
Subgroup B_2	1	7770	5050	8760	8800	
	2	5190	7710	8500	10870	3819
	3	3419	4920	10040	10820	4815
	4	5576	10800	13200	8310	5320
	5	3439	4380	4630	6770	2474
	Average	5078	6572	9026	9114	4107
Subgroup N	6	2340	4550	7300	6550	3340
	7	4010	7870	8380	4120	3400
	8	4500	9740	8320	10290	3790
	9	4730	8720	8210	8250	4590
	10	7740	7890	7890	7058	4220
	Average	5064	7754	8020	7253	3868
Subgroup K	11	3310	5330	7200	8653	4400
	12	4940	6610	8060	10450	3970
	13	3200	8060	8500	9175	7930
	14	5680	5770	6800	6240	2160
	15	4750	8700	9510	9448	3870
	Average	4376	6894	8014	8793	4466

* See Footnote to Table III.

though in a slight degree[13], which is due to a disturbed utilization of aminoacids in these children.

Effect of riboflavine or nicotinamide upon the excretion of aminoacid nitrogen in the 24 hour-urine of children with nutritional dystrophy.

The results were given in Tables III–VII (Cf. Tables III–VII). The average values of the urinary aminoacid nitrogen in Subgroups B_2, N and K were shown in Fig. 1 (Cf. Fig. 1). The average values of aminoacid nitrogen in Subgroup B_2 decreased markedly on the 18th day of the ingestion of riboflavine and remained then within the normal range of the urinary aminoacid nitrogen, and gradually dropped slightly below the normal range on the 72nd day of riboflavine administration. In Subgroup K, the average value of the urinary aminoacid nitrogen remained above the normal range till the 42nd day, then falling into the normal

TABLE VI

The Percentage of Aminoacid Nitrogen to Total Nitrogen in the 24 Hour Urine of Subgroups B_2, N and K

Subgroup	No. of case*	Percentage of aminoacid-N to total-N				
		Before	Days of administration of riboflavine or of nicotinamide			
			8 days	19 days	42 days	72 days
Subgroup B_2	1	3.0	3.0	0.9	0.8	
	2	3.4	2.5	0.8	0.5	0.4
	3	6.3	5.6	1.6	1.8	2.1
	4	2.9	1.6	0.8	0.9	1.5
	5	4.0	3.4	1.0	1.4	1.5
	Average	3.9	3.2	1.0	1.0	1.3
Subgroup N	6	2.9	2.4	0.5	2.1	0.6
	7	3.5	1.6	0.4	3.3	0.6
	8	4.6	1.9	1.0	1.9	1.4
	9	4.3	2.2	1.6	2.0	0.8
	10	2.6	2.2	1.3	2.1	1.9
	Average	3.5	2.0	0.9	2.2	1.0
Subgroup K	11	5.4	3.3	1.8	1.6	1.2
	12	2.9	1.6	1.7	1.2	1.9
	13	3.8	1.5	1.3	1.8	1.4
	14	3.4	4.5	2.1	1.5	2.9
	15	3.7	1.8	1.3	1.4	3.4
	Average	3.8	2.5	1.6	1.5	2.1

* See Footnote in Table III.

range on the 72nd day. In Subgroup N, the average value of the urinary aminoacid nitrogen showed a marked fluctuation and dropped below the normal range on the 72nd day of nicotinamide administration. From the results above mentioned, it will be said that, on the basis of the average values of the urinary aminoacid nitrogen in Subgroups B_2, N and K, the most marked and definite decrease was observed in Subgroup B_2 and that riboflavine deficiency was, as in case of riboflavine deficiency in rats reported by Schweigert *et al.*[10], one of factors contributing to an increased output of the urinary aminoacid nitrogen in the children with nutritional dystrophy.

But as will be seen in Case 3 of Subgroup B_2 (Cf. Table IV), there was a case in which the urinary aminoacid nitrogen remained above the normal range in spite of a successive administration of riboflavine for 42

TABLE VII

The Ratio of Aminoacid Nitrogen (mg%) to Creatinine Nitrogen (mg%) in the 24 Hour Urine of Subgroups B_2, N and K

Subgroup	No. of case*	Ratio of aminoacid-N (mg.%) to creatinine-N (mg.%)				
		Before	Days of administration of riboflavine or of nicotinamide			
			8 days	19 days	42 days	72 days
Subgroup B_2	1	1.28	1.18	0.80	0.79	
	2	1.40	1.64	0.55	0.44	0.21
	3	2.46	2.57	1.26	2.04	0.99
	4	1.21	1.27	0.75	0.83	0.69
	5	2.04	1.99	0.68	1.27	0.82
	Average	1.67	1.73	0.80	1.07	0.67
Subgroup N	6	1.00	1.28	0.42	1.07	0.26
	7	1.41	1.19	0.34	0.98	0.23
	8	1.55	1.38	0.67	1.66	0.57
	9	1.71	1.42	0.96	1.30	0.36
	10	1.15	1.27	0.96	1.22	0.67
	Average	1.36	1.30	0.67	1.24	0.41
Subgroup K	11	1.98	1.89	0.96	1.11	0.53
	12	1.67	1.83	1.46	1.40	0.99
	13	1.17	1.09	0.84	1.26	0.59
	14	1.47	3.27	1.22	1.30	1.07
	15	1.52	1.58	1.23	1.09	2.29
	Average	1.56	1.93	1.14	1.23	1.09

* See Footnote in Table III.

days. This finding suggested that an increased output of the urinary aminoacid nitrogen was not entirely to be ascribed to riboflavine deficiency alone in the children with nutritional dystrophy.

Now how will the decrease in the urinary aminoacid nitrogen in Subgroup K—in the Subgroup with no medication whatever—on the 72nd day of the observation (July 1st, 1953) be explained? A possible explanation for this finding may be given by 'seasonal variation of the incidence in riboflavine deficiency.' Masuda *et al.*[14], basing upon the clinical observations throughout a whole year on the inhabitants in this District, found that the riboflavine concentration in blood was the lowest during the period from April to May, and that an incidence in clinical manifestations of ariboflavinosis was the highest during the period from May to June. This observation of Masuda is very interesting to me,

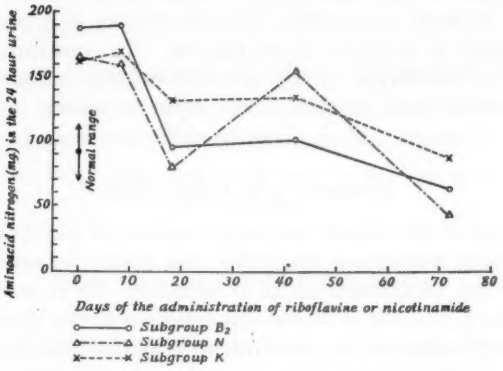

Fig. 1. Average values of vrinary aminoacid nitrogen in Subgroups B_2, N and K.

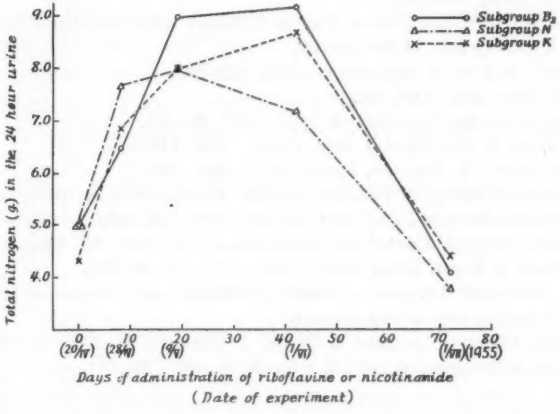

Fig. 2. Total nitrogen in the 24 hour urine of subgroups B_2, N and K.

because the urinary excretion of total nitrogen in our children with nutritional dystrophy increased markedly during the period from April to May, as is shown in Fig. 2, and because, according to Pollack *et al.*[15], an increase in catabolism would cause a considerable loss of riboflavine from the body into the urine. The 72nd day of my own observation was July 1st, by this time the urinary excretion of total nitrogen had decreased markedly (Cf. Fig. 2), showing a subsidence of the catabolism which must have been increased during the period from April to June. From the end of June on, in addition to a subsidence of the catabolism as mentioned

above, the intake of riboflavine would be naturally increased owing to a consumption of green vegetables. The riboflavine deficiency was, therefore, ameliorated in its degree from July on. In consequence of such an improvement of riboflavine deficiency, the urinary output of aminoacid nitrogen, provided that its increased output is mianly due to this deficiency, was decreased and ought to be decreased from July on.

Summary and Conclusions

An increase in the urinary output of aminoacid nitrogen was found in the children with nutritional dystrophy and it was, in general, markedly reduced after an administration of riboflavine. So it seemed that riboflavine deficiency was one of factors contributing to the disturbance in the aminoacid metabolism in the children with nutritional dystrophy.

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Riboflavine Concentration of the Biopsied Livers from Children with or without Nutritional Dystrophy

Studies on the Nutrition of Children in Hirosaki Area

(47th Report)

By

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The signs of riboflavine deficiency, such as angular stomatitis, cheilosis and swelling of the lingual papillae, were frequently found among children in Hirosaki Area, and these ariboflavinotic signs have been accepted as part manifestations of nutritional dystrophy[1]. In the preceding paper[2], Kagaya, one of us, reported that the blood level of riboflavine was, in general, lower in children with nutritional dystrophy than in those without it.

In the present investigation riboflavine concentration in the liver specimens biopsied from children with or without nutritional dystrophy will be dealt with.

Experimental

Method and Material

The liver biopsy was carried out in the same way as described in Sato's report[3]. Adhering blood was blotted from the biopsied specimens, and 5–10 mg. of them were used for the estimation of riboflavine. The extraction of riboflavine from the liver tissues was performed according to Yagi's procedure[4] and riboflavine was determined by lumiflavin-fluorometric method[5),6].

The histological preparations of the biopsied liver and the description of their microscopic findings were done in the same way as described in Sato's report[1].

The children subjected to this investigation were the patients admitted into our Clinic. 20 of these children had shown clinical signs of riboflavine deficiency. The rest, 10 in number, had been free from such symptoms.

Results and Comment

In the biopsied liver specimens from the 20 cases with nutritional dystrophy, the average for the total riboflavine concentration was 20.8± 2.4 γ per gram, and that for the esterified form was 12.8±1.5 γ per gram, corresponding to 61.5% of the total riboflavine. In the biopsied liver specimens of the 10 children without nutritional dystrophy, the average for the total riboflavine was 28.2±7.1 γ per gram, and that for the esterified form was 16.6±3.2 γ per gram, being 58.9% of the total riboflavine (Cf. Table I and Fig. 1).

On the microscopic examination, the plant cell-like appearance of

TABLE I

Riboflavine Concentration in the Biopsied Liver Specimens of Children with or without Ariboflavinotic Signs

No. of case	Name and sex	Age (years and months)	Method for nutrition in infancy	Signs of rivoflavine deficiency			Liver palpable below the costal margin (cm.)	Clinical diagnosis
				Swelling of lingual papillae	Angular stomatitis	Cheilosis		
1	O.K. ♀	4.0	Mother's milk	##	−	−	4.5	Nutritional dystrophy
2	T.M. ♀	3.1	Dried milk	##	−	+	0.5	Mongolian idiocy
3	A.T. ♂	1.1	Dried milk+rice flour	##	−	+	3.0	Rickets
4	K.R. ♀	0.6	Rice flour+dried milk	##	−	−	3.0	Nutritional dystrophy
5	N.Y. ♂	0.2	Mother's milk+rice flour	++	−	−	4.5	Enlargement of the liver
6	N.N. ♀	5.2	Mother's milk+ cow's milk	++	+	−	0.5	Nutritional dystrophy
7	H.S. ♀	0.6	Dried milk	−	−	−	0.5	Rhinopharyngitis
8	H.K. ♂	5.1	Mother's milk+rice flour	##	+	−	1.5	Mongolian idiocy
9	N.K. ♂	0.9	Mother's milk	−	−	−	0.5	Tuberculosis of hiliar limph nodes
10	S.Y. ♂	1.8	Dried milk	−	−	−	1.5	Diaphragmatic hernia
11	I.M. ♂	2.1	Dried milk	−	−	−	1.5	Rickets

12	M.F. ♀	4.0	Mother's milk+dried milk	++	−	−	7.0	Pick's polyserositis
13	Y.S. ♂	5.2	Mother's milk+dried milk	++	++	−	3.0	Mongolian idiocy
14	N.K. ♀	5.1	Mother's milk	+	++	++	1.5	Tuberculosis
15	N.T. ♂	2.0	Mother's milk+dried milk	++	+	++	2.0	Nutritionol dystrophy
16	S.K. ♂	3.9	Mother's milk	−	−	−	5.0	Enlargement of the liver
17	M.T. ♂	6.1	Mother's milk	++	−	−	3.5	Nutritional dystrophy
18	M.S. ♂	2.6	Mother's milk	+	−	+	2.0	Tuberculous meningitis+Nutritional dystrophy
19	K.Y. ♀	4.9	Mother's milk	++	−	−	3.0	Mongolian idiocy
20	M.K. ♀	0.6	Mother's milk	−	−	−	3.0	Rhinopharyngitis
21	K.Y. ♀	1.0	Mother's milk+rice flour	++	−	+	3.0	Atrophy
22	K.Y. ♀	1.1	Mother's milk+dried milk	++	−	−	6.0	Atrophy
23	K.Y. ♀	1.2	Dried milk	++	−	−	4.0	Atrophy
24	K.T. ♀	0.4	Dried milk+rice flour	++	+	+	3.0	Miliary tuberculosis
25	K.T. ♀	0.5	Dried milk	−	−	−	4.0	Miliary tuberculosis
26	K.T. ♀	0.8	Dried milk	−	−	−	3.0	Miliary tuberculosis
27	K.H. ♂	0.7	Dried milk+rice flour	−	−	−	2.0	Atrophy
28	K.H. ♂	0.8	Dried milk	−	−	−	3.0	Atrophy
29	S.I. ♂	1.4	Dried milk+rice flour	++	−	+	3.0	Nutritional dystrophy
30	S.I. ♂	1.4	Dried milk+rice flour	+	−	+	1.0	Nutritional dystrophy

the hepatic cells[3] was found in 13 cases (Cases 1–4, 6, 8, 11, 13, 17, 19, 21, 26 and 27), and in these cases, the average for the total riboflavine concentration was $18.9\pm3.3\,\gamma$ per gram and that for the esterified form was $11.7\pm2.3\,\gamma$ per gram, being 61.9% of the total riboflavine. The plant cell-like appearance of the hepatic cells was not found in 12 cases (Cases 5, 7, 9, 10, 15, 16, 22, 24, 25, 28, 29 and 30), and in these cases, the average for the total riboflavine was $27.0\pm\gamma6.1\,\gamma$ per gram and that for the esterified form was $16.7\pm2.5\,\gamma$ per gram, being 61.9% of the total

TABLE IB

Riboflavine Concentration in the Biopsied Liver Specimens of Children with or without Ariboflavinotic Signs

No. of case*	Histological changes of liver				Riboflavine concentration in the liver		
	Hepatic cells			Portal fibrosis	Total (γ/g)	Esterified (γ/g)	Ratio of esterified to total riboflavine (%)
	Plant cell-like appearance	Fatty infiltration	Atrophy				
1	++	−	−	−	12.9	12.5	96.9
2	+	−	−	−	20.0	8.3	41.5
3	+	+	−	+	13.6	9.8	72.0
4	+	−	−	++	21.0	10.9	51.9
5	−	−	−	++	15.5	14.0	90.3
6	++	++	+	−	21.2	15.7	74.1
7	−	−	−	−	18.9	17.7	93.7
8	+	−	−	−	18.9	14.8	78.3
9	−	−	−	−	30.0	26.6	88.7
10	−	−	−	−	28.1	18.8	66.9
11	++	−	−	−	20.1	10.8	53.7
12					21.1	12.8	60.7
13	+	−	−	+	19.1	15.2	79.6
14					32.3	15.6	48.3
15	−	−	++	−	24.3	12.0	49.4
16	−	−	++	++	22.4	14.5	64.7
17	+	−	−	−	12.4	8.1	65.3
18					23.2	11.0	47.4
19	++	−	−	−	18.1	16.2	89.4
20					28.2	16.0	56.7
21	++	−	−	−	15.6	3.8	24.4
22	−	−	−	−	27.3	14.4	52.7
23					22.5	13.0	57.8
24	−	−	−	−	24.5	15.9	64.9
25	−	−	−	−	27.1	20.0	73.8
26	+	−	−	+	33.0	11.2	33.9
27	++	−	−	−	20.3	15.1	74.3
28	−	−	−	−	53.6	15.2	28.4
29	−	−	−	−	30.9	15.9	51.5
30	−	−	−	−	22.2	15.9	71.6

* No. of case was the same as in Table I A.

riboflavine (Cf. Table I and Fig. 2). These results showed that the concentration of the riboflavine, in both total and esterified forms, tended to

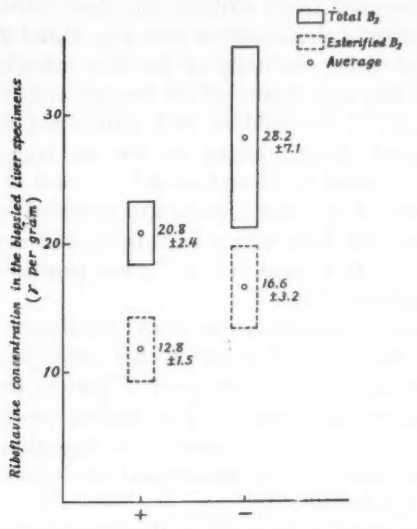

Fig. 1. Riboflavine concentration in the biopsied liver speciments of children with or without ariboflavinotic signs.

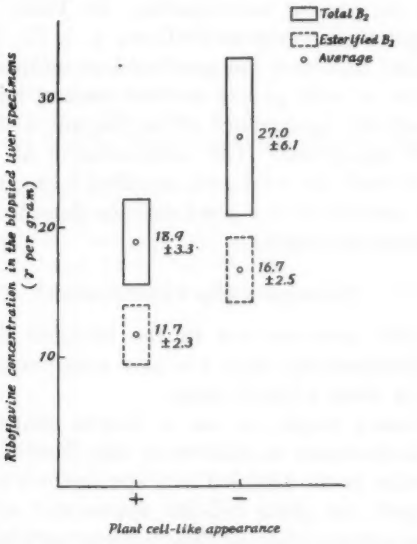

Fig. 2. Riboflavine concentration in the biopsied liver specimens with or without plant cell-like appearance.

be higher in the biopsied livers without the plant cell-like appearance of the hepatic cells than in those with it (Cf. Fig. 1 and 2).

Sato[3] reported from the study of his own materials of liver biopsy that the plant cell-like appearance of the hepatic cells was observed in 22 cases out of 30 cases of the children with nutritional dystrophy and that this histopathological change might be due to high-carbohydrate-low-protein diet. As reported by Masuda *et al.*[7], in most of the inhabitants in this Area, about 60% of the caloric intake was provided with highly polished rice; vegetable and fish were scanty in variety, and eggs, milk and meat were scarcely taken. In a word, that is " a low protein-high-carbohydrate and B vitamin deficient diet."

Respecting the relationship between riboflavine metabolism and protein depletion, Sarett *et al.*[8] reported that concentration of riboflavine in the liver varied directly with the level of protein in the diet and was independent of the vitamin intake. This finding was later confirmed by Unna *et al.*[9], who showed that, in spite of the ingestion of liberal amount of riboflavine, the livers of rats maintained on a low protein diet were unable to retain riboflavine.

The clear relationship between a decreased concentration of riboflavine in the biopsied liver specimens and poor protein intake was observed from Table II, in which only the infants under 12 months were abstracted from the data of the present investigation. In Table II, the cases with ariboflavinotic signs were found in 5 (Cases 4, 5, 21, 24 and 27), all of which were those fed mainly on rice gruel with an addition of small amount of milk preparation or with poorly secreted human milk, and in 3 cases of which plant cell-like appearance of the hepatic cells was observed in the biopsied liver specimens. The concentration of riboflavine in the liver specimens, in both the total and esterified forms, tended to be lower in the infants fed mainly on rice gruel than in those fed on mother's milk or milk preparations exclusively.

Summary and Conclusions

The riboflavine concentration in the biopsied liver was lower in children with ariboflavinotic signs (as part manifestations of nutritional dystrophy) than in those without same.

Protein deficiency might be one of factors contributing to the development of ariboflavinosis in children in this District, because the riboflavine concentration in the biopsied liver specimens tended to be lower in those cases in which the plant cell-like appearance of hepatic cells was histologically demonstrated than in those without such histological changes.

TABLE II

Relation among Ariboflavinotic Signs, Riboflavine Concentrationin the Biopsied Liver Specimens and Methods for Nutrition in Infants under 12 months

Method for nutrition	Mother's milk			Milk preparation			Mainly rice gruel with an addition of small amount of mother's milk or milk preparation		
Concentration of B_2 in liver	No. of case	B_2 in liver (γ/g)		No. of case	B_2 in liver (γ/g)		No. of case	B_2 in liver (γ/g)	
		Total	Esterified		Totol	Esterified		Total	Esterified
	9	30.8	26.6	7	18.9	17.7	(4)*	21.0	10.9
	10	28.2	16.6	25	27.1	20.0	(5)	15.5	14.0
				26*	33.0	11.2	(21)*	15.6	3.8
				28	53.6	15.2	(24)	24.5	15.9
							(27)*	20.3	15.1
	Average	29.1 ± 14.9	21.3 ± 95.1	Average	33.1 ± 23.6	16.0 ± 6.1	Average	19.4 ± 4.6	11.9 ± 6.2

* The cases in which plant cell-like appearance of the hepatic cells was demonstrated by liver biopsy. ( ) The cases with ariboflavinotic signs.

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Histopathological Findings on a Function of Bronchiolus respiratorius

By

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It has been many times discussed that dusts are deposed on the walls of the bronchioli respiratorii (Br. resp.), but the mechanism of such deposition has been left unelucidated.

We have examined the picture of dust accumulation in normal human lungs, and have succeeded in ascertaining that the inhaled dusts are absorbed neither via the alveoli belonging to the sacculi alveolares nor the wall itself of the Br. resp., but via the alveoli projecting from the wall of the latter (Fig. 1). The air exchange in such alveoli belonging to the Br. resp. during respiratory movement is apt to deteriolate, so that dust particles, it is understood, penetrate through the alveolar walls in the region and are finally caughted up by the so-called lymphoid tissue in the walls of Br. resp.

This findings is of importance, not only in extending the study on the mechanism of dust accumulation in the lung, but also in suggesting the first stage of the development of inflammation and tumors frequently starting from the Br. resp.

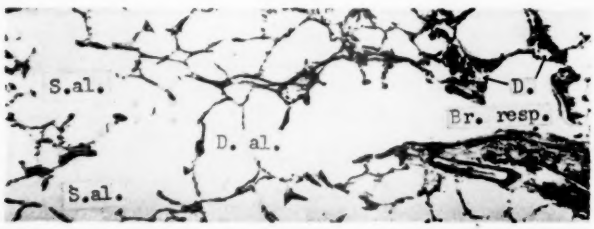

Fig. 1. Dusts are present in the alveoli projecting from the Br. resp., but not in the alveoli belonging to the S. al. Br. resp.—Bronchiolus respiratorius, D. al—Ductus alveolaris, S. al—Sacculus alveolaris, D.—Dusts.

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Experimental Studies on the Coronary Insufficiency and the Coronary Occlusion

I. On the Relation of the Coronary Blood Flow and the ST-deviation in the Electrocardiogram

By

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It is widely known that when the coronary blood supply does not fulfil the oxygen demand of the myocardium the deviation of the ST-segment in the electrocardiogram occurs. However, the quantitative studies, that is, the determination of the extent of the reduction of the coronary flow which causes the ST-deviation, have not been carried out in detail. As such a study in the literature we find only the work of Wégria[1]. He inserted the cannula into the anterior descending branch of the left coronary artery and diminished the flow by partial occuluding of the screw clamp which was set between the artery and the flowmeter. The observed findings in the electrocardiogram, however, were the elevation of the ST-segment. In this respect, Wégria's finding is considered to be somewhat differed from the situation in the usual coronary insufficiency. It is needless to say that the ST-segment usually depersses in the clinical coronary insufficiency.

The reason why the ST-segment elevated in his experiment is hard to be explained. In his experiments, the anterior descending branch was prepared out from the cardiac mass in order to facilitate the insertion of the cannula. During this manipulation, in our opinion, the artery may be compressed almost totally in some instants, so that the temporary transmural ischemia may occur. In such a situation the sole reduction of the flow by partial occluding of the screw clamp may be apt to cause the transmural ischemia and the ST-elevation may appear as its manifestation. In this viewpoint, we attempted to examine the ST-changes when

the flow was diminished in the unexposed left coronary artery.

Experimental

Methods

The dogs weighing 6.0 kg. to 20.5 kg. were employed. Under pentobarbital anesthesia and the artificial respiration the chest wall as well as the pericardium were cut open and the heart was exposed. After the preparation of the brachiocephalic artery the cannula connected with the flowmeter was inserted into it. Then the cannula was advanced via the ascending aorta and wedged in the orifice of the left coronary artery.

Measurement of the coronary flow. The bubble flowmeters which have the capacity of 2 cc., 4 cc., or 6 cc. were used. The other end of the flowmeter was connected to the left femoral artery. The outline of the procedures was illustrated in Fig. 1.

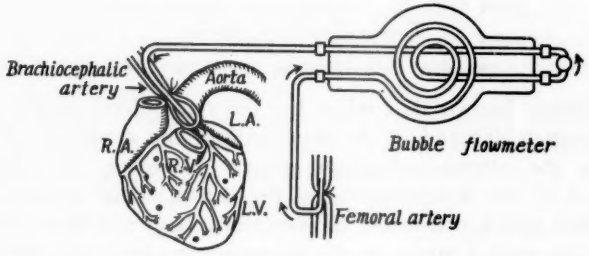

Fig. 1. The outline of the experimental procedure. The black circles on the cardiac surface indicate the location of the electrodes.

The electrocardiographic leads. The electrocardiograms were recorded by I, II, III, and the direct unipolar leads from the ventricular surface: the location of the electrodes were shown also in Fig. 1. The central terminal was employed as the indifferent electrode.

The methods to reduce the coronary flow. We employed following procedures: (1) Spontaneous diminution of the flow. (2) Compression of the rubber or vinyl tube connecting the flowmeter to the arteries. (3) Intravenous injection of the hypotonics, such as C_6, Rauwolfia serpentina, histamine, etc.

Cannula. Among above methods the second may be the most preferable, because, analogous to the clinical coronary insufficiency in the coronary sclerosis, there are no remarkable changes in the arterial blood pressure. But, when the tube is compressed, the blood sometimes flows directly into the coronary artery from the aorta via the space between the

coronary arterial wall and the cannula, since the aortic pressure remains high. In order to stop this direct flow from the aorta we used occasionally the cannula with a sponge fragment at its bend, or the cannula with a balloon at its head, which is expanded with the air blowed through the steel tube attached along the cannula.

Results

1) *Observations when the coronary flow diminished spontaneously.* The coronary flow was measured in 19 dogs in which the flow diminished spontaneously as the result of " weakening " of the heart. In every cases the ST-depression was observed when the coronary flow diminished, as shown in Fig. 2. In this figure the white circles indicate the absence and the black circles the presence of the ST-depression. The shaded circles indicate the questionable depression.

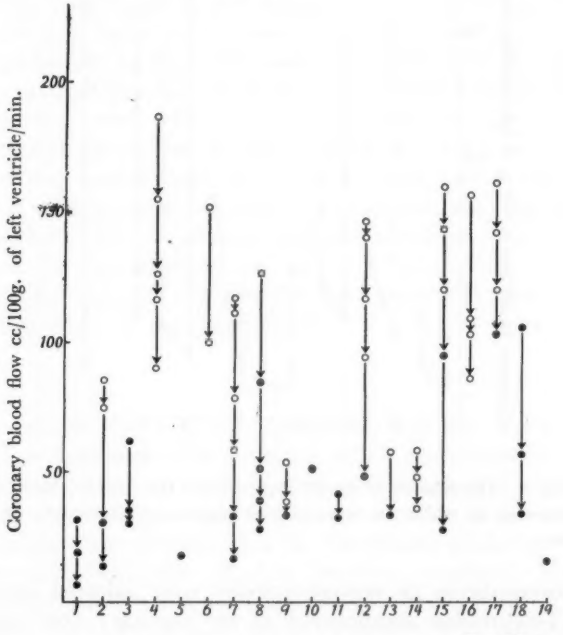

Fig. 2. The relation of the ST-depression to the coronary blood flow.
(1) The cases of spontaneous blood pressure fall. Details in text.

The level of the coronary flow which causes the ST-depression was not uniform in individual cases. In some dogs the ST-depression appeared

when the flow was above 100 cc./100 g. of left ventricle/min., while in others the ST-segment was not depressed even at the level of 40 cc. Generally speaking, the unquestionable depression appeared when the coronary flow was below 50 cc./100 g. of left ventricle/min., hence this value may be regarded as a critical or threshold level for the ST-depression. The ST-depression in Case 18, which was observed at the level of 106 cc. may be attributed to the myocardial damage due to the operative manipulation, because it was present even immediately after opening of the chest. This case was excluded, therefore, from the consideration.

2) *Observations when the hypotonics were used.* The intravenous injection of the hypotonics lowered the arterial blood pressure as well as diminished the coronary flow. As the coronary flow diminished, the ST-depression came out (Fig. 3). In these hypotonic states the hypertonics

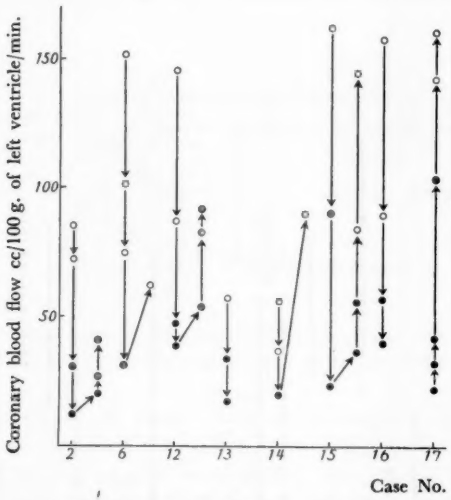

Fig. 3. The relation of the ST-depression to the coronary blood flow. (2) The cases in which the hypotonics or hypertonics were used. Details in text.

such as neosynephrine or nor-epinephrine were injected intravenously, then the ST-depression disappeared as the coronary flow augumented. The electocardiograms in one case are shown in Fig. 4. In these observations the critical levels of the flow which caused the ST-depression were also different in individual cases, but 50–60 cc./100 g. of left ventricle/min. may be regarded as such a level.

3) *The normal value of the coronary blood flow.* The initial value of the

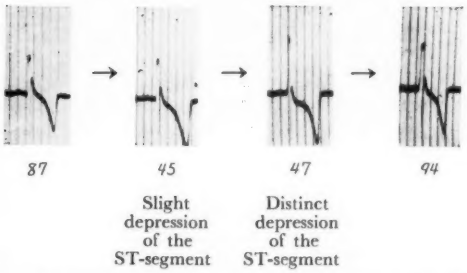

Fig. 4. The relation of the coronary blood flow to the ST-depression in the direct unipolar lead from the surface of the left ventricular apex. The numbers shown below the records indicate the coronary blood flow, cc/100 g of left ventricle/min.

coronary flow was varied in individual cases. Some of the dogs show the considerably low value even immediately after opening of the chest. It may be attributed to the inadequate arrangement of the operative procedures. In most experiments in the better conditions the coronary flow was measured as nearly 150 cc./100 g. of left ventricle/min. at the beginning of the observations, hence we may regard this value as normal in the open-chest dog anesthetized with pentobarbital under artificial respiration. This value is relatively high in comparison with the data given by the various authors[2)-15)]. . Most authors described the values below 100 cc. The reason for the difference is obscure. Foltz, *et al.*[11)] and Goodale, *et al.*[11)] pointed out that the pentobarbital anesthesia may be a cause of the augumentation of the coronary flow.

Discussion

From above results it was concluded that the diminution of the coronary flow below 50 to 60 cc./100 g. of left ventricle/min. produces the ST-depression. The reversibility of the ST-depression observed in the augumentation of the coronary flow caused by the hypertonics supports the conclusion. The opinion, that the diminution of the flow is the cause of the electrocardiographic changes, has been commonly accepted since long years ago. But it has not been proved directly. Most authors conducted their conclusion from the pathological[16)-18)] or clinical[19)-21)] findings, it might say, indirectly.

The critical value for the ST-depression is considerably low in comparison with the normal value. There is a range of nearly 60% reduction of the flow which does not cause the ST-depression. This range may be regarded as a safety zone. The relation is diagrammatically illustrated in

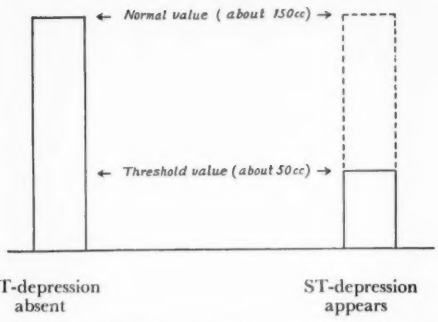

Fig. 5. Diagrammatical representation of the threshold value of the coronary blood flow for the ST-depression.

Fig. 5. But it may be different in various conditions. For instance, it may be not applied to the cases in which the myocardial oxygen demand increases, such as in the physical exercise, because it is probably accompanied by the augumentation of the coronary flow. Hence another critical level must be found. The conclusion, that 50–60 cc./100 g. of left ventricle/min. is the critical level for the ST-depression, is applied only for the conditions as examined in our experiments.

From these experiments it is ascertained that the reduction of the coronary flow is the cause of the ST-depression. But, in general view, the mechanism to depress the ST-segment is not the diminution of the coronary flow itself, but the myocardial oxygen defficiency resulted from it. Therefore, the further considerations regarding this relation are necessary.

We are not competent for the discussion on this point because we have not measured the oxygen content of the coronary arterial and venous blood. According to Gregg[9], the myocardial oxygen consumption of the anesthetized open-chest dogs is 8–10 cc./100 g. of left ventricle/min. Since the oxygen content of the arterial blood is nearly 18 vol %, the coronary flow of 45–55 cc./100 g. of left ventricle/min. may fulfil the oxygen demand of the myocardium. This value of the flow fairly coincides with the critical level obtained in our experiments.

There remains, however, some problematic points in above considerations. In the first place, this value was calculated on the supposition that, when the flow diminished below the critical level, the whole oxygen contained in the coronary arterial blood were spent in the myocardium and the oxygen content in the coronary vein were zero. But it is improvable fact, because the oxygen content in the venous blood cannot be lower than in the myocardium. In this respect, the critical value must be higher than above mentioned value. Secondly, the oxygen demand of the

myocardium may decrease in the hypotonic states such as in our experiments. In this standpoint, the value of 8–10 cc. may be regarded somewhat too high.

Accordingly, above calculations are certainly not correct. These two conditions, however, are supposed to counteract with each other, hence we consider that some clues regarding the problems have been obtained.

At any rate, from these observations we can confirm the current view that the nature of the coronary insufficiency is the defficient supply of the oxygen from the coronary artery for the myocardium.

Summary

1. The critical or threshold value of the coronary blood flow for the ST-depression was measured in 19 dogs by the bubble flowmeter connected to the cannula inserted into the orifice of the left coronary artery.

2. The normal value was measured as 150 cc./100 g. of left ventricle/min. in the open-chest dog anesthetized with pentobarbital under artificial respiration.

3. As the coronary flow reduced, the ST-depression appeared. This critical value of flow was measured as 50–60 cc./100 g. of left ventricle/min.

4. The ST-depression disappeared reversibly when the coronary flow was augumented by the intravenous injection of the hypertonics in the hypotonic states.

5. By means of approximate calculation the coronary flow of this level fairly coincides with the lowest value to fulfil the myocardial oxygen demand.

6. From above considerations, the current view, that the reduction of the coronary flow or the defficient oxygen supply for the myocardium is the cause of the ST-depression, is verified directly.

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Experimental Studies on the Coronary Insufficiency and the Coronary Occlusion

II. The Relation of the Coronary Blood Flow to the Arterial Blood Pressure, and the Effect of the Vagus Nerves upon It

By

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In the previous report[1] we used the hypotonics such as C_6, Rauwolfia, etc. in order to diminish the coronary flow. As seen in our experiments the coronary flow diminishes when the arterial pressure falls. According to the literature, however, the relation between them is not so simple. Various opinions were published regarding this problem.

Relatively large numbers of the authors have the opinion that the coronary blood flow diminishes accompanying with the fall in the arterial blood pressure. Already in 1913 Markwalder and Starling[2] and Morawitz and Zahn[3] introduced such an opinion. From that time, Hochrein and Keller[4], Schmidt and Engelhorn[5], Klisiecki and Fleck[6], and others[7][8][9] pointed out the dependence of the coronary flow on the arterial blood pressure, although it is, of course, not considered as a sole factor. Rein[10], however, claimed that the factor to change the coronary flow is the oxygen requirement of the heart muscle, but not the blood pressure. According to him, the coronary blood flow is dependent on the arterial blood pressure only in the enfeebled, dilated hearts, or under the intravenous barbiturate anesthesia. Gollwitzer-Meier and Kroetz[11], Foltz, *et al.*[12], Eckstein, *et al.*[13], and others[14] also stated that the coronary blood flow changes rather in the response to the myocardial oxygen consumption.

On the one hand, the function of the vagus and sympathetic nerves must be taken into account concerning this problem, because, as widely known, the coronary vessels are distinctly influenced by them. But some of the authors, especially who thought the role of the myocardial oxygen consumption as most important, denied the effect of the nerves on this relation.

In this respect we have analyzed our data obtained in the experiments reported in the preceding paper, in which the vagus nerves were intact in some cases and cut off in others.

Experimental

Methods

All the procedures were same as in the previous paper. The blood flow in the left coronary artery was measured by the bubble flowmeter and the blood pressure in the femoral artery was recorded by the mercury manometer. The employed hypotonics were C_6, Rauwolfia serpentina, or histamine, etc., and as the hypertonics, neosynephrine and nor-epinephrine were chosen. Both vagal trunks were cut in the cervical region.

Results

The observations were classified in three groups: 1) in the cases of the spontaneous fall in the arterial blood pressure, 2) in the cases of the blood pressure fall caused by the hypotonics, and 3) in the cases of the blood pressure rise caused by the hypertonics injected in the hypotonic states resulted from above two conditions. The results were summarized in Figs. 1, 2, 3, 4, 5 and 6, in which the values of the coronary flow were plotted against the arterial blood pressure.

It was observed that in the cases of the spontaneous blood pressure fall the coronary flow diminished in general with the lowering of the pressure, as seen in Figs. 1 and 2. This finding was seen whether the vagus nerves were intact or not.

On the other hand in the cases in which the hypotonics were used such a relation was hard to be observed, as shown in Fig. 3. But after cutting both cervical vagi it seemed to be present (Fig. 4). In order to investigate these relations exactly, we have calculated the correlation rates between the arterial blood pressure and the coronary flow. The results were summarized in Table I.

The significant correlation were observed in the cases of the spontaneous blood pressure fall, though it was not so high, while in the cases of the hypotension introduced by the hypotonics it was not present when the vagus nerves were intact. After cutting vagi, however, the correlation rate became somewhat higher. Similar results were obtained in the cases in which the hypertonics were injected in the hypotonic states. The rate was very low in the intact vagal cases, while considerably high in the severed vagal cases.

Discussion

We have two subjects to study on the relation between the arterial

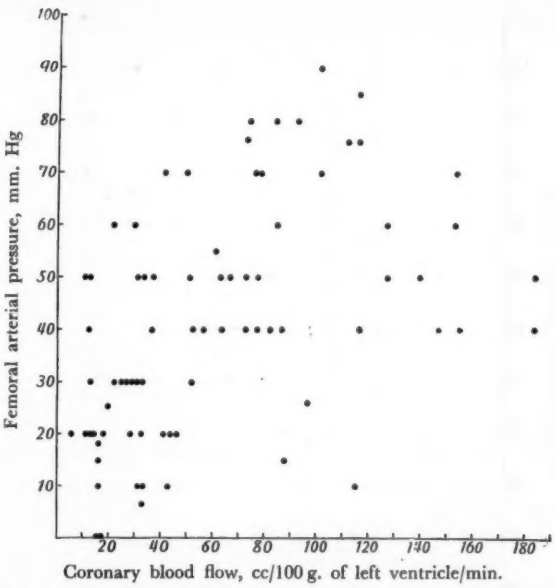


Fig. 1. The relation of the coronary blood flow to the arterial blood pressue. (1) Spontaneous blood pressure fall in intact vagal cases.

blood pressure and the coronary flow: One is the change in the coronary flow when the arterial blood pressure is primarily changed, and the other is the change in the arterial blood pressure when the coronary flow is primarily changed. These two situations, however, may be not regarded as the same. The main purpose in this paper is to study the former problem.

As already mentioned, there is a disagreement between the authors regarding the relation of the coronary flow to the arterial pressure. Some authors observed the presence of the direct dependence of the former on the latter, while others denied it. We believe, our data will make some contributions to compromise this disagreement of the opinions.

Our experimental observations disclosed that the vagus nerves have a role on this relation in some measure. The correlation rates were higher, as a rule, in the severed vagal cases than in the intact vagal cases. The vagus nerves are frequently compared to a safeguard equipment against the derangement in the living body. It is supposed, in this viewpoint, that the vagus nerves play role on the maintenance of the coronary flow when the arterial pressure falls critically.

In the cases of the spontaneous blood pressure fall the correlation was

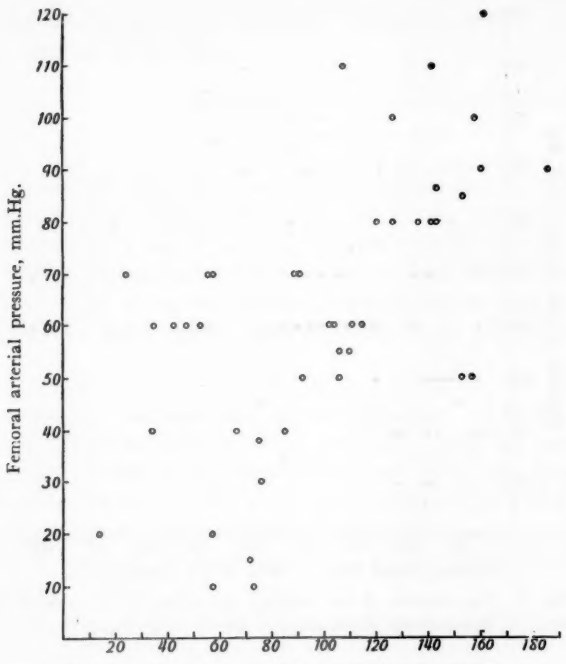


Fig. 2. The relation of the coronary blood flow to the arterial blood pressure. (2) Spontaneous blood pressure fall in vagi cut cases.

significant even when the vagi were not cut. This is explained by the idea that in such cases the function of the vagi was poor as a result of " weakening " of the heart. From these considerations, Rein's view that the coronary blood flow is influenced by the arterial blood pressure only in the enfeebled heart can be explained by the lowering of the function of the vagi.

Some authors pointed out that the coronary flow is ruled by the cardiac oxygen consumption. Their conclusion, however, were obtained in the cases of the intact vagi for the most part. Nevertheless, already in the old literatures the effects of cutting vagi were examined. More than about twenty years ago, Anrep and Segall[15] reported that the arterial pressure has effect on the coronary flow only in the vagus-cut cases. Gollwitzer-Meier, *et al.*[11] observed similar findings. Probably because of some reason, this finding was neglected and in the recent papers the role of the vagus nerves has come again into question.

Fig. 3

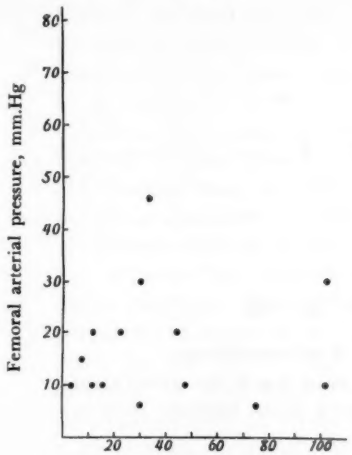


Fig. 4

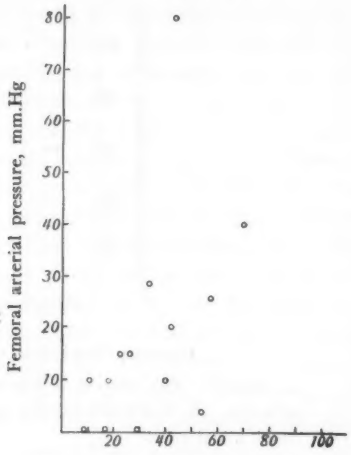


Fig. 3. The relation of the coronary blood flow to the arterial blood pressure. (3) When hypotonics were used in intact vagal cases.

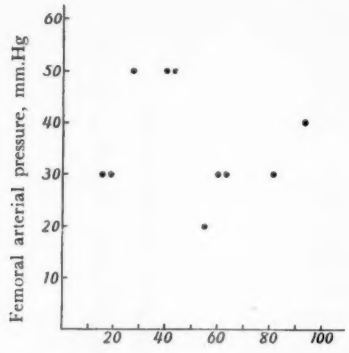
Fig. 4. The relation of the coronary blood flow to the arterial blood pressure. (4) When hypotonics were used in vagi-cut cases.

TABLE I

Correlation Rates between the Coronary Blood Flow and Arterial Blood Pressure

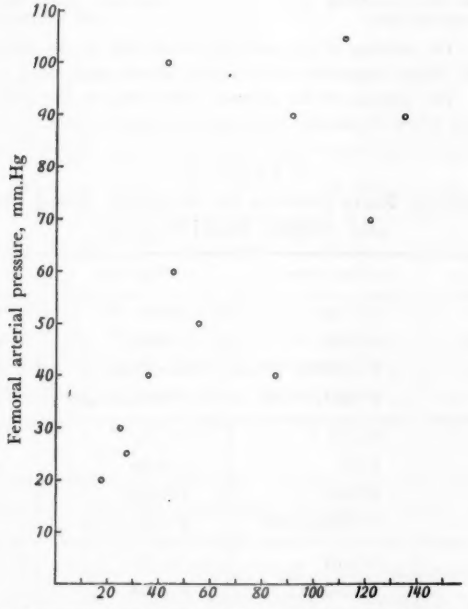
	Vagi intact	Vagi cut	Total
Spontaneous blood pressure fall	N=78 r=0.50 Fs=25.33 F (0.01)=6.96	N=43 r=0.69 Fs=37.25 F (0.01)≒7.30	N=121 r=0.61 Fs=69.89 F (0.01)=6.84
When hypotonics were used	N=15 r≒0 Fs≒0 F (0.05)=4.69	N=15 r=0.49 Fs=4.11 F (0.05)=4.67	N=30 r=0.21 Fs=1.29 F (0.05)=4.20
When hypertonics were used	N=10 r=−0.19 Fs=0.30 F (0.05)=5.32	N=12 r=0.68 Fs=8.52 F (0.05)=4.96	N=22 r=0.58 Fs=10.13 F (0.01)=8.10

Our analysis of the correlation showed that the pressure-flow relation-



Coronary blood flow, cc/100 g. of left ventricle/min.

Fig. 5. The relation of the coronary blood flow to the arterial blood pressure. (5) When hypertonics were injected in the hypotonic states in intact vagal cases.



Coronary blood flow, cc/100 g. of left ventricle/min.

Fig. 6. The relation of the coronary blood flow to the arterial blood pressure. (6) When hypertonics were injected in the hypotonic states in vagi-cut cases.

ship approached to the linear one to some extent when the vagi were cut. Osher[16] pointed out that this relation is rather logarithmic, that is, log-pressure to flow relation is linear. Such a relation, however, was not obtained in our data. The reason for the difference may be attributed to the difference in the experimental procedures. Osher used the preparations of doner and recipient dogs, and the perfusion pressure of the flow from the former's heart were variously changed. In our opinion, Osher's observations may be more suitable in the biological sense, but ours also has some significances, because it means that the arterial pressure tends to influence directly on the coronary flow if the vagal function is abolished.

Whether the sympathetic nerves play part on this relation or not, is also a problem. But we have not studied on it. At the start of our experiments reported in the preceding paper, we had no special purposes to study the role of the vagus nerves, and the vagi were cut merely for the elevation or maintenance of the arterial pressure. Analyzing the data the results shown in Table I was obtained. It is, therefore, no more than a by-product, and the role of the sympathetic nerves did not come into our considerations.

The problem discussed in above section is the change in the coronary flow caused by the primary change in the arterial pressure. There is another problem, as already mentioned, how is the behavior of the arterial pressure when the coronary flow is changed primarily. We have also carried out some experiments on it; the changes in the arterial pressure was examined when the coronary flow was reduced by compressing the rubber or vinyl tube connecting the flowmeter to the brachiocephalic artery. But as shown in Fig. 7, it showed no changes either in the intact vagal or in the severed vagal cases.

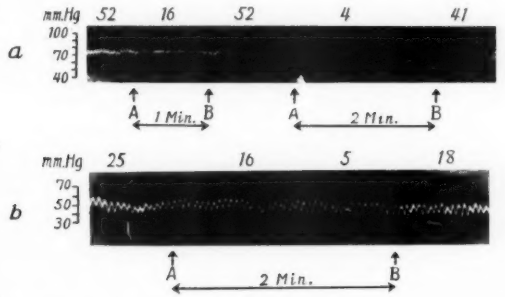


Fig. 7. Records of the arterial pressure when the coronary blood flow was primarily changed. (a) Before and (b) after section of the vagi. At (A) the tube was compressed, and at (B) released. The numbers above the records indicate the coronary blood flow.

There are few reports on the experimental reduction of the coronary flow. We can find only the works of Wégria[17], Katz, *et al.*[18], Rein[10], and others. Wégria's work was concerned the ST-depression as referred in our preceding paper. The experiments of Katz, *et al.* were to study the reactive hyperemia after the temporary reduction of the coronary flow in the fibrillating heart. Only the experiments of Rein, who published the data that the arterial pressure fluctuates in parallel with the change in the coronary artery flow when the heart became enfeebled, have the relation to the problem discussed here. But we have never observed such a situation even in the cases with markedly low pressure and reduced coronary flow. Fig. 8 shows one of our observations.

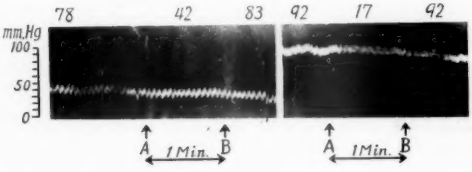


Fig. 8. Records of the arterial pressure when the coronary blood flow was primarily changed. The left is the case which shows the lower initial pressure, and the right the higher initial pressure. Both show little changes in the arterial pressure in spite of the marked changes in the cornary blood flow.

It is a confused problem to answer why no changes in the arterial pressure are observed when the coronary flow is primarily reduced. But it is at least sure that the mechanism to maintain the arterial pressure when the coronary flow reduces is not related to the vagus nerves, because its severing has no effect. Presumably, the reduction of the coronary flow does not directly or immediately result in the weakening of the myocardial contraction. Of course, however, if the excessive reduction are continued too long, the ventricular fibrillation occurs. Nevertheless, its occurance is usually arbupt and the blood pressure shows little change until that time.

Summary

1. The relation of the coronary blood flow to the arterial blood pressure was analyzed using the experimental data reported in the preceding paper. In general, the fall of the blood pressure was accompanied by the diminution of the coronary flow.

2. As a rule, the correlation rates were higher in the severed vagal cases than in the intact vagal cases.

3. In the spontaneous blood pressure fall the correlation was significant even in the intact vagal cases, though it is not so high. This may be attributed to the lowered function of the vagi as a result of " weakening " of the heart.

4. From above observations it was supposed that the vagus nerves act to maintain the coronary flow when the arterial pressure falls critically; that is to say, they act as a safeguard equipment.

5. On the other hand, the arterial pressure showed no changes when the coronary flow primarily reduced. The vagus nerves played no part on it.

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Stomach Cancer and ABO Blood Groups

By

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Recently, the correlation between cancer or other diseases and the ABO blood groups has come again into attention of the medical scientists.[1),2),3),4)]

The authors of this paper have made a comparative study of the distribution of the blood groups of stomach cancer patients with that of the general population of Japan, referring ourselves on the data furnished by many university hospitals in this country. As shown in Table I, among the 1,385 cancer cases, blood group A was found in 41.2%, B in 21.4%, O in 29.7% and AB in 7.7%. In comparison with the blood group distribution in the general Japanese Population,[5)] we found that the percentage of A group was significantly larger and that of AB group was significantly smaller among the cancer patients than among the general populace, at the level of significance of 5%.

TABLE I

		Total	A	B	O	AB
Stomach cancer	Number	1,385	571	296	412	106
	%	100.0	41.2	21.4	29.7	7.7
General population	Number	530,046	203,255	115,416	161,461	49,914
	%	100.0	38.3	21.8	30.5	9.4
Significance of difference			*	–	–	*

* Denotes significance at the 5% level.

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Biochemical Studies on Carbohydrates

CCII. Synthesis of D-Galactose-4-β-D-galactoside and D-Glucose-6-β-galactoside

By

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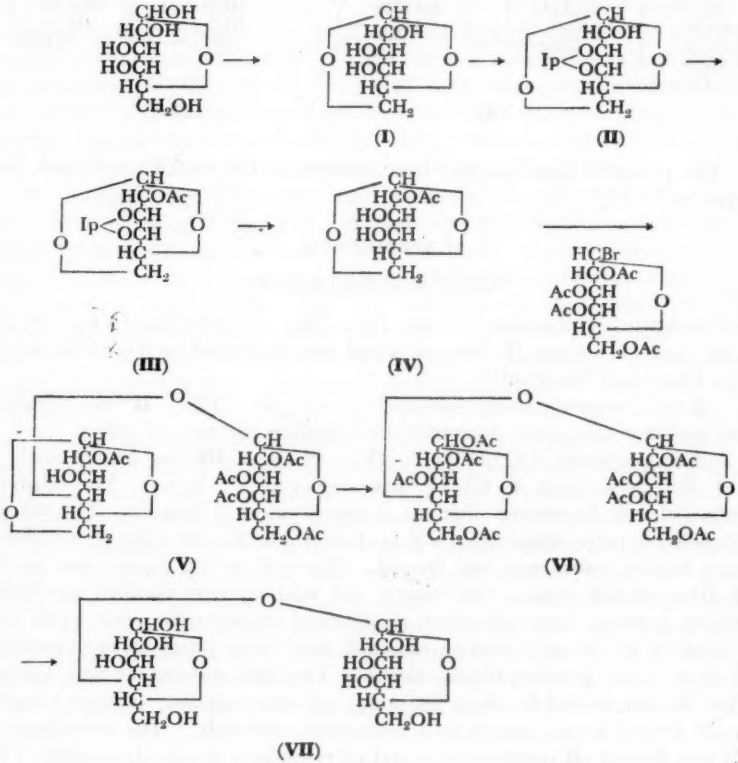
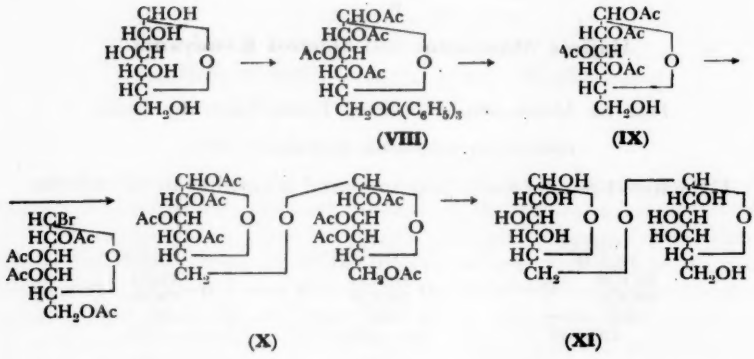
Galactose-4-β-galactoside was prepared according to the scheme:



The product (amorphous) melted at 186°C and had $[\alpha]_D^{27}$ (water) of +52.7°. That C atom 4 of one of the galactose residue in it is the position where C atom 1 of the other is attached was substantiated by determining the amount of periodate consumed by the β-methylglycoside which had been prepared from it through the heptaacetyl-bromo derivative.

Synthesis of glucose-6-β-galactoside was tried by following up the stages:



The product (amorphous) here melted at 167–168°C and had $[\alpha]_D^{28}$ (water) of +37.5°.

Experimental

Galactose-4-β-galactoside

3,4-Isopropylidene-galactosan<1,5>β<1,6> (**II**). Commercial lactose was pyrolyzed and galactosan (**I**) thus obtained was condensed with acetone according to Hann and Hudson.[1]

2-Acetyl-3,4-isopropylidene-galactosan<1,5>β<1,6> (**III**). **II** was acetylated with pyridine and acetic anhydride by following the authors above.

2-Acetylgalactosan<1,5>β<1,6> (**IV**). 2.5 g. of **III** was heated with 25 cc. of 20% acetic acid at 100°C under reflux for 2½ hours. The substance dissolved clearly 15 minutes after the commencement of heating. The solution once cooled to room temperature was heated for a while with some active charcoal in a boiling water-bath and filtered. The bulk on the funnel was washed with 10 cc. of hot water. The filtrate and washing were distilled together in vacuo to a syrup, then submitted to repeated evaporations with water until the smell of acetic acid disappeared, and freed from water by reevaporations with three 10 cc. protions of abs. alcohol. The final still-residue was dissolved in 2 cc. of abs. alcohol by slight warming, and after standing at room temperature for several hours, placed in a refrigerator overnight. The crystalline deposit was filtered off under suction and recrystallized twice. It weighed 1.2 g.

and melted at 115°C.

2-Acetylgalactosan-4-β-(tetraacetyl)-galactoside (**V**). 1.02 g. of **IV**, 2.5 g. of sliver oxide[2], 5.0 g. of calcium sulfate which had been ignited in an electric furnace for two hours and allowed to cool in a desiccator, and 10 cc. of purified chloroform were shaken together in a stoppered brown flask, and added to with 0.3 g. of crystalline iodine at one time and with 5 cc. of chloroform containing 2.05 g. of acetobromogalactose[3] in small portions. The mixture was further shaken for 50 hours, during which small portions of the mixture were taken out at intervals to test their filtrates for bromide, filtered through charcoal with suction and washed with about 20 cc. of chloroform. The filtrate and washing were united and distilled in vacuo, and the still-residue was distilled with dry benzene several times and placed in a vacuum-desiccator ($CaCl_2$).

Octaacetylgalactose-4-β-galactoside (**VI**). 1) Opening of the anhydro ring in **V**. 2.4 g. of it was taken up in 40 cc. of a 7:3 (by volume) mixture of acetic anhydride and glacial acetic acid and cooled on ice, and 0.2 cc. of conc. sulfuric acid was added under continuous cooling. After standing on ice for 4 hours, the reaction mixture was poured in small portions into a mixture of 20 g. of crystalline sodium acetate and 200 g. of chopped ice, whereby an oily deposit occurred. When the ice thawed, the mixture was nearly neutralized with sodium carbonate (powder) and exhausted with three 50 cc. portions of chloroform. The chloroformic solutions combined were washed with water, dried with anhydrous calcium chloride and distilled under reduced pressure. The yellowish syrupy still-residue was dried in vacuo over $CaCl_2$. Yield 2.1 g.

2) Separation of **VI** by the chromatographic procedure of McNeely *et al.*[4] 100 g. of a Magnesol-Celite (5:1) mixture was packed into a 2.3×19.5 cm. column with suction and wetted with 20 cc. of dry benzene (thiophene free). And the mixed acetylation products above were dissolved in 10 cc. of the benzene and poured onto the column. The chromatogram was developed with 800 cc. of a mixture of the benzene and abs. ethanol in the volume proportion of 100:1. The column was extruded and streaked with a 1% solution of potassium permanganate in 10% NaOH. Differently colored bands were indicated at regions 0–0.3 cm. (I), 5.5–7.4 cm. (II), 11.4–13.1 cm. (III) and 17.2–19.5 cm. (IV) from the top, and in particular Bands I and IV were distinctly colored. Band I was rejected. Bands II–IV, whose upper and lower 1 cm. wide margins were cut off, were separately extracted with 200–300 cc. of purified acetone, and the solutions were distilled to syrupy consistency. The Band II extract was diluted with 95% ethanol and concentrated, when it changed into a mush of polygonal crystals. These crystals were filtered off and recrystallized from abs. ethanol. 392 mg. of crystals in thin prisms were given. The product here was recrystallized 4 times from 96% ethanol until it melted at a constant temperature of 172–173°C. $[\alpha]_D^{25}$ (chloroform) $= +1.161 \times 100/1 \times 2.027 = +57.3°$. The syrupy extract of Band III amounted little, and that of Band IV and the effluent gave together 1.2 g. of probable crude pentaacetylgalactose (thin needles of F.P. of 92–93°C).

Galactose-4-β-galactoside (**VII**). 150 mg. of **VI** was dissolved in 4 cc. of

hot dry methanol and cooled to room temperature. It was added to with 1 cc. of 0.1 *N* sodium methylate, and stood at room temperature for 2 hours, stoppering tightly. Then the reaction mixture was diluted with 10 cc. of water, passed through a 1.1×5.2 cm. (5 cc.) column of Amberlite IR-120 (H-form) and washed with 70 cc. of water. The effluent and washing were distilled under reduced pressure to a syrup, taken up in 10 cc. of water and decolorized with 0.1 g. of charcoal, followed by condensation to a syrup. Distillation with ethanol was processed three times. The final still-residue was dissolved in 2 cc. of abs. methanol, and 4 volumes of ether were added. The occurring white precipitate was centrifuged off, submitted to dissolution and precipitation twice more in a similar manner, and after washing with ether, dried in vacuo over P_2O_5. A white hygroscopic powder given here amounted to 70 mg. It sintered at 183°C and melted at 185–186°C. $[\alpha]_D^{31}$ (water) $= +0.52 \times 100/1 \times 0.985 = +52.7°$. Iodine use[5] as glucose reached 51.17%. Paper chromatography was effected by the aid of Solvent butanol-pyridine-water after Masamune and Yosizawa[6]. The chromatogram showed a single spot of Rr of 0.29.

Heptaacetyl-β-methylgalactosido-4-β-galactoside. 800 mg. of **VI** obtained after several runs was dissolved in 2.4 cc. of glacial acetic acid and cooled in iced water. The solution was added to with 1.8 cc. of glacial acetic acid saturated with HBr, and after standing for 30 minutes under continuous cooling, transferred to a separatory funnel containing 50 cc. of iced water and 50 cc. of chloroform. The mixture was shaken energetically and stood. The chloroformic layer was separated, washed with changes of ice-chilled water until it showed no acid reaction to BCG and dried with anhydrous calcium chloride. It was then distilled at room temperature under reduced pressure to perfect dryness. 683 mg. of a non-colored substance was given. F.P. 87–91°C. $[\alpha]_D^{31}$ (chloroform) $= +1.67 \times 100/1 \times 2.045 = +81.7°$.

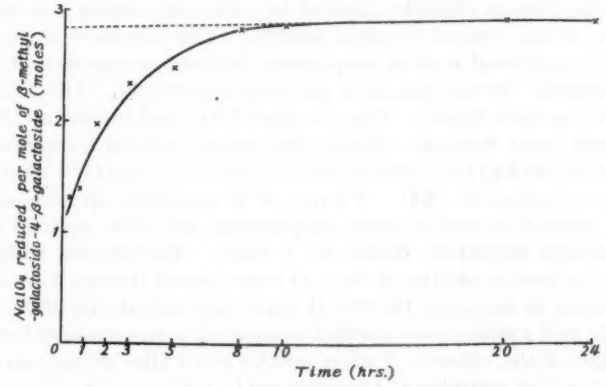
650 mg. of it was taken up in 15 cc. of abs. methanol, shaken for 24 hours together with 1.0 g. of silver oxide and 3 g. of $CaSO_4$ previously ignited and filtered with suction. The bulk on the funnel was washed with abs. methanol. The brownish filtrate, whose bromide test resulted in negative, and the washing were distilled, and the dry still-residue was dissolved in 5 cc. of abs. alcohol. The solution was bubbled with H_2S-gas. The turbidity that occurred was removed by means of a small amount of charcoal, and the clear filtrate was condensed and dried in vacuo over P_2O_5. The substance given (425 mg.) was dissolved in 5 cc. of dry pyridine, and acetylated with 5 cc. of acetic anhydride, shaking for 5 hours and subsequently standing for 31 hours. The reaction mixture colored somewhat brown was distilled at low temperature, and the still-residue was distilled many times with ethanol and benzene to expel off the last trace of pyridine and placed in a vacuum desiccator containing anhydrous calcium chloride.

It was taken up in 10 cc. of benzene and fractioned by column chromatography by the aid of 50 g. of a mixture of Magnesol and Celite as above[4]. The size of the column was 2.5×13.5 cm. The volume of the solvent for irrigation was 1 *l*. The alkaline permanganate indicated three bands at regions 0–0.3 cm.

(I), 1.5–2.6 cm. (II) and 8.0–12.5 cm. (III) from the top of the column. Bands II and III were extracted with 200 and 400 cc. of purified acetone respectively, and the solutions were concentrated separately to syrupy consistency. The extract of Band II showed the negative Fehling reaction, but that of Band III the positive Fehling reaction. The former extract was dissolved in a mixture of ethanol and benzene. On concentration of the solution, it changed into a white crystalline porridge. The crystals were filtered off and recrystallized from abs. ethanol three times, and 136 mg. of colorless crystals in needles was given. They showed constant melting point of 186°C. $[\alpha]_D^{25}$ (chloroform) $= -0.492 \times 100/1 \times 1.464 = -33.1°$.

β-Methylgalactosido-4-β-galactoside. 160 mg. of heptaacetyl-β-methylgalactosido-4-β-galactoside was dissolved in 5 cc. of 0.02 *N* sodium methylate and stood at room temperature for 2 hours. The solution which became turbid was added to with 20 cc. of water and passed through a column of 5 cc. of Amberlite IR-120 like the one above. The effluent and washing were condensed to a syrup and dissolved in 3 cc. of abs. methanol, and 4 volumes of dry ether were added. The deposit was reprecipitated, washed twice with dry ether and placed in a vacuum desiccator containing P_2O_5. 80 mg. of an uncolored amorphous powder was given. It was hygroscopic. F.P. 164–166°C. $[\alpha]_D^{25}$ (water) $= +0.085 \times 100/1 \times 1.2 = +7.1°$.

Fig. 1



Metaperiodate oxidation of β-methylgalactosido-4-β-galactoside. 32.5 mg. of the product above was dissolved in 5 cc. of 0.1 *M* acetate buffer pH 5.0, and after standing in a water-thermostat at 5–6°C for a while, added to with 1 cc. of 0.4201 *M* sodium metaperiodate at the same temperature. 0.5 cc. portions were pipetted out at intervals from the mixture to estimate the oxidant remaining unreduced as described by Masamune and Yosizawa[7]. The consumption of the periodate increased with time along the curve in Fig. 1 and reached 2.8 moles per mole of the substrate, which are nearly in accord with the calculated value of 3 for the structure assigned.

Glucose-6-β-galactoside

6-Trityl-1,2,3,4-tetraacetylglucose (**VIII**). It was prepared by adapting Reynolds and Evans modification[8] of Helferich and Klein method. F.P. 166°C.

1,2,3,4-Tetraacetylglucose (**IX**). Reynolds and Evans' description[8] was followed. F.P. 122°C.

Octaacetylglucose-6-β-galactoside (**X**). A minor modification of the method of Reynolds and Evans[9] for preparing gentiobiose octaacetate was applied, replacing Drierite with commercial calcium sulfate previously ignited. 2.6 g. of 1,2,3,4-tetraacetylglucose, 2.0 g. of silver oxide[2], 7.5 g. of the calcium sulfate and 15 cc. of dry chloroform were shaken together for 1 hour in a stoppered brown flask and added to with 0.25 g. of solid iodine at first and afterwards with five 2 cc. portions of chloroform containing 3.0 g. of acetobromogalactose in all, each addition being followed by vigorous shaking. The mixture was further shaken for 48 hours, filtered through a carbon layer with suction and washed with chloroform. The filtrate which was free from bromide and the washing were distilled at 25°C to a syrup and redistilled twice with 30 cc. of abs. ethanol. The final still-residue was dissolved in 2 cc. of abs. ethanol and poured into 200 cc. of ice-chilled water under agitation. The white amorphous precipitate changed granular on continued agitation. After standing in an ice-box overnight, it was filtered off under suction and dried in air for two days. It was next dissolved in 200 cc. of ether, and the solution was dried by the aid of anhydrous calcium chloride, distilled in vacuo to a syrup and then taken up in 30 cc. of abs. ethanol by slight heating. This solution was concentrated to about 5 cc. and stood at room temperature, whereupon crystals in thin needles began to appear. It was placed in an ice-box overnight. The crystals were brought on a suction funnel. They weighed 2.9 g. and metled at 155–161°C. Recrystallized twice from abs. ethanol, the product showed a constant melting point of 162°C and had $[\alpha]_D^{28}$ (chloroform) of $+20.73°$ ($=+0.217° \times 100/1 \times 1.047$).

Glucose-6-β-galactoside (**XI**). 300 mg. of **X** was taken up in 10 cc. of hot methanol, allowed to cool to room temperature, and after addition of 2.5 cc. of 0.1 *N* sodium methylate, shaken for 3 hours. The solution which turned turbid was clarified by addition of 30 cc. of water, passed through a 1.1×10.4 cm. (10 cc.) column of Amberlite IR-120 (H-form) and washed with 50 cc. of water. The effluent and washing were distilled in vacuo to a syrup and redistilled with three changes of abs. ethanol. A white powder given after drying was dissolved in abs. methanol by warming and precipitated by addition of 4 volumes of ether. The white deposit here was centrifuged off, washed with ether twice and dried in vacuo over $CaCl_2$. Yield 142 mg. F.P. 167–168°C. $[\alpha]_D^{28}$ (water) $=+0.388 \times 100/1 \times 1.035 =+37.5°$. Iodine use as glucose[5], 52.97% (Calcd., 52.63%). A solution (1%) in water and a 1 *N* H_2SO_4-hydrolysate of the amorphous product were paper-chromatographed with Solvent butanol-pyridine-water (5:3:2 by volume) according to Masamune and Yosizawa[6]. The former gave a single spot of an Rr of 0.23 and the latter two spots at the positions corresponding to glucose and galactose.

A portion of product was dissolved in a small volume of methanol, and after addition of abs.

ether to slight cloudiness, stood in a desiccator containing $CaCl_2$ (ordinary pressure). Globular crystals settled after a while. However, they were so hygroscopic that separation of them was given up.

The expenses of this work were defrayed by a grant given from the Education Department through the Grant Committee for Scientific Researches. H. Masamune.

References and Notes

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2) Treated beforehand according to Helferich and Klein (Liebigs Ann. Chem., 1926, **450**, 219).
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Cancer of Cervix Uteri and ABO Blood Groups

By

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The authors have studied in comparison the distribution of ABO blood groups among patients of cancer of cervix uteri and among the general population in Japan, referring ourselves to the data furnished by many university hospitals in all parts of the country. As shown in Table I, among the 1,534 cancer cases, 41.7% belonged to group A, 19.8% to group B, 29.1% to group O and 9.4% to group AB. In comparison with the group distribution of the general populace,[1] the percentage of group A cases was found significantly larger among the cancer patients at the level of significance of 1%.

TABLE I

		Total	A	B	O	AB
Cancer of cervix uteri	Number	1,534	640	304	446	144
	%	100.0	41.7	19.8	29.1	9.4
General population	Number	530,046	203,255	115,416	161,461	49,914
	%	100.0	38.3	21.8	30.5	9.4
Significance of difference			*	–	–	–

* Denotes significance at the 1% level.

A similar study was made with breast cancer cases as subject. It was found that of the 207 patients, 36.0% belonged to A blood group, 19.6% to B group, 34.7% to O group and 9.7% to AB group. Here, no significant difference from the general distribution of blood groups was found in any group, but the percentage of O group cases among the cancer cases was considerably larger than among the general population,[1] so that it seems predictable that a significant difference may be found, when more breast cancer cases are tested for their blood group in the future.

References. 1) Kobayashi, Hanzai-gaku Zasshi (Jap.), 1940, **14**, 727.

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Biochemical Studies on Carbohydrates

CCIII. A Glucidamin and a Molisch-positive Mucopolysaccharide from Human Lung

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In 1949, Utusi[1] separated from pig lungs two glucidamins, glacial acetic acid-soluble and -insoluble, besides heparin as hexosamine-containing compounds. And, in 1954, Glegg *et al.*[2] demonstrated chromatographically the presence of bound galactose, glucose, mannose and L-fucose in a fraction of a cattle lung extract without regard to the homogeneity of the fraction. That fraction was free from glucuronic acid and contained no less than 10% nitrogen.

The present account deals with a similar study on human lungs. Recently Akama[3] collected lungs of Group A persons and those of Group O persons separately to investigate group lipids in them. Most of the materials employed by the writers were the tissue residues which had been exhausted with cold acetone and ether and a hot methanol-chloroform mixture in succession by Akama. Resembling materials were prepared and put together with them according to the group of persons. The united materials will be referred to as Material A′ and O′. A glucidamin and a Molisch-positive mucopolysaccharide have been separated from each of the materials. The glucidamin from Material A′ was found specifically Group A active though weak, but the corresponding glucidamin from Material O′ proved not only Group O but also Group A and B active. Group interconversion *in vitro* probably occurred in the latter group glucidamin which is also very weak (Cf. Akama[3]). The mucopolysaccharides were group inactive. As to the quantitative composition as well as optical rotation, the two glucidamins also differed from each other but the mucopolysaccharides from the two sources were identical.

Experiments and Comments

Preparation Procedure

The case started with Material A′ will be described in detail.

400 g. of Material A′ was powdered in an iron mortar and shaken with 5 volumes of 10% NaCl (A few drops of octylalcohol were added to avoid foam formation) for 24 hours at room temperature (5–15°C). The centrifugate was submitted to extraction 4 times more, and the solutions were united and filtered. The filtrate (yellowish brown, somewhat turbid) was dialyzed against running water for 3 days, distilled in vacuo to about 100 cc. and saturated with $(NH_4)_2SO_4$. After standing overnight, the precipitate was centrifuged off, dissolved in 80 cc. of water and precipitated again by saturation with $(NH_4)_2SO_4$, followed by centrifugation. The supernatant fluids were mixed together, dialyzed for 4 days against running water, and after distilling in vacuo to about 1/10 the volume, again dialyzed for two days. They were then condensed to a syrup and precipitated with an excessive abs. alcohol. The deposit was washed with alcohol and dried in vacuo over H_2SO_4. A white powder weighing 1.0 g. was given—**Fr. I.**

The final precipitate by $(NH_4)_2SO_4$ above was taken up in 80 cc. of water, dialyzed for 5 days and distilled to a syrup, which was precipitated with 100 cc. of abs. alcohol, washed with abs. alcohol and dried—**Fr. II_1.** 1.5 g. was the yellowish powder given here.

Tests of the fractions resulted as follows.

	Molisch	Goldschmiedt	Biuret	Bial-Mejbaum	Dische	P
Fr. I	++	++	++	++	+	+
Fr. II_1	++	+	+	±	±	+

To free from the Goldschmiedt-positive mucopolysaccharide and nucleic acids, the whole balance of Fr. II_1 was dissolved in 100 cc. of water and warmed to 37–38°C, and 50 g. of anhydrous sodium sulfate was added in small portions. After standing in an incubator at 34°C for 3 hours, the deposit was separated on a filter under continuous warming. It was subjected to three repetitions of the dissolution and precipitation, employing each time 80 cc. of water and 40 g. of anhydrous sodium sulfate. The filtrates were combined, diluted with water and dialyzed against running water for 3 days. They were followingly condensed, precipitated and washed with abs. alcohol. The substance given was dried and set aside—**Fr. II_2.** The precipitate at Na_2SO_4 concentration of 33% showed intensive Molisch reaction but neither Bial nor Dische reaction, and was free from phosphorus. It was further fractioned to separate off the part precipitable at Na_2SO_4 concentration of 22%. With the aim, 100 cc. of water and 28 g. of Na_2SO_4 were used at first and in the three repeated treatments 50 cc. of water and 14 g. of Na_2SO_4. The temperature of the incubator was maintained at 26°C. The fraction that was recovered from the mother fluids similar to above was 0.5 g.—**Fr. II_3.** The final precipitate was also dialyzed and recovered. 0.25 g. was the dry brown powder given here (**Fr. II_4**).

Fr. II_2 was dissolved in 100 cc. of water and shaken with 50 cc. of 80% (w/w) phenol. The Molisch-positive ingredients remained in the watery layer, which was distilled and precipitated with alcohol. The substance given dissolved in 0.2% ammonium acetate (pH 5.0) and precipitated mostly on addition of lead acetate, suggesting pentosenucleic acid, and Fr. II_4 was almost Molisch-negative. In other words, Fr. II_3, which was Molisch ++, Goldschmiedt ± and biuret ++, was thought to contain a glucidamin or glucidamins.

Hence, another portion weighing 250 g. of Material A′ was again fractioned and the corresponding Frs. II_3 were united. This mixture (1 g. in total) was taken up in 200 cc. of distilled water, and after rejecting the part insoluble, acidified to pH 3.8 with dilute HCl, whereby slight turbidity occurred. The turbid solution was placed in a refrigerator overnight and filtered. The clear, yellowish filtrate was electrodialyzed, and some precipitate occurring was discarded after centrifugation. The supernatant was distilled under reduced pressure to syrupy consistency, and excessive abs. alcohol containing some sodium acetate was added. The deposit was separated, washed with abs. alcohol and dried. A white powder weighing 200 mg. was given. It gave Molisch as well as biuret reaction markedly but showed two boundaries after electrophoresis in acetate buffer pH 4.6, I 0.2, so that it was fractionally precipitated with alcohol to isolate the glucidamin or glucidamins in view. At first, it was dissolved in 20 cc. of water, and 40 cc. of abs. alcohol was added in portions. To the centrifuged supernatant (The small precipitate was scarcely Molisch positive) was added a large volume (150 cc.) of additional alcohol. Since, however, even opalescence did not happen, the mixture was adjusted to pH 4.6 by the aid of 20% acetic acid. Thereupon a flocculent precipitate appeared, which was separated in a centrifuge after standing overnight in a refrigerator. It was again taken up in 10 cc. of water. pH of the solution was 5.6. After rejecting a tiny insoluble part, the solution was added to with 15 cc. of abs. alcohol, and the precipitate which was only faintly Molisch positive was also removed. The solution was next acidified to pH 4.6, stood in a refrigerator for 2 hours and centrifuged. The centrifugate was washed with alcohol and dried to a constant weight in vacuo over H_2SO_4—**Glucidamin A.** 92 mg. of a white powder were yielded.

Isolation of the mucopolysaccharide. In the second run of fractionation (with 250 g. of Material A′), 1 g. of a substance corresponding to Fr. I above was obtained. Those two corresponding substances were submitted together to the procedure of Homan and Lens[4] as follows. They were taken up in 200 cc. of water, and after freeing from the small insoluble part, electrodialyzed. The solution decreased to 180 cc. It was made up to the original volume, adjusted to pH 7.2 with dilute NaOH and shaken with 100 cc. of 80% (w/w) phenol on a shaker for 30 minutes, followed by standing overnight at room temperature (6°C). Centrifuged. The watery layer was siphoned out (The substance recovered from the phenol layer was Molisch ± and Goldschmiedt −) and repeatedly distilled in vacuo to syrupy consistency, adding water from time to time. The final syrup was precipitated with abs. alcohol, and after washing

with changes of abs. alcohol, dried. The yield at this stage was 400 mg. To eliminate the contaminating nucleic acids, the product was dissolved in 130 cc. of 0.2% ammonium acetate and adjusted to pH 5.0 with acetic acid, and 15 cc. of a 10% solution of crystalline lead acetate, previously adjusted to pH 5.0 with acetic acid, were added in drops until no more increase of turbidity. After standing in a refrigerator overnight, the small precipitate was discarded. To the clear mother fluid (140 cc.) was added 80 cc. of abs. alcohol. The centrifuged supernatant which was Goldschmiedt-negative was distilled to about 100 cc. and dialyzed for 3 days, when Pb was not detected anymore. It was distilled in vacuo to a syrup and precipitated with abs. alcohol. The deposit was separated, washed with abs. alcohol and dried to a constant weight in vacuo over H_2SO_4. The white powder given—**Mucopolysaccharide I**—amounted to 200 mg.

From 780 g. of Material O′ were prepared 120 mg. of a glucidamin—**Glucidamin O**—and 202 mg. of a mucopolysaccharide—**Mucopolysaccharide II**—in a manner similar to above.

Properties of the Products

Electrophoresis. The preparations proved homogeneous or nearly homogeneous in electrophoresis in acidic and alkaline buffers (Fig. 1).

Solubility. Mucopolysaccharide I and II behaved similar to each other. They were insoluble in glacial acetic acid and 90% phenol, and were not precipitated from water by ammonium sulfate, cupric chloride, lead acetate and baryta.

The glucidamins were found soluble in glacial acetic acid and 90% phenol, insoluble in the sat. ammonium sulfate and sat. sodium sulfate (33°C) and precipitable from water by cupric chloride and lead acetate.

Blood group assay. The assay was processed on a glass plate as described by Masamune *et al.*[5] The mucopolysaccharide preparations were neither Group A nor B nor O active. The results on the glucidamins are illustrated in Table I. Glucidamin A was Group A active specifically, though weak. Glucidamin O was weakly Group O active but also Group A and B active besides, probably due to group interconversion during preparation, that is, the polysaccharide moiety acting as the determinant group might have been laterally fixed in part to the amino acid grouping with which it is bound N-glycosidically (Cf. Akama[3], Masamune and Akama[6] and Masamune *et al.*[7]).

Test-tube tests. The results are shown in Table II.

Paper partition chromatography of the sugar and amino acid components. Sugars. 10 mg. substance was dissolved in 1 cc. of 1 *N* H_2SO_4 and hydrolyzed in a sealed tube at 100°C for 5 hours. When cold, the hydrolysate was neutralized to pH 5.8 with the sat. baryta. The deposit was centrifuged off and washed. The supernatant and washing were distilled together to about 0.1 cc., and 0.02 cc. portions were applied on paper slivers (Toyo Roshi filter paper No. 2) to develop chromatograms with solvents butanol-pyridine-water (5:3:2 by volume) and *n*-

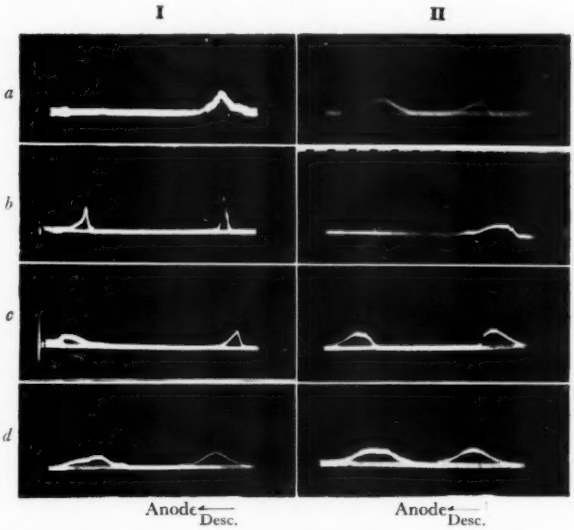

Fig. 1. Electrophoretic patterns (2/3×) of 1% solutions of Glucidamin A (*a*) and O (*b*) and Mucopolysaccharide I (*c*) and II (*d*) in acetate buffer of pH 4.6 and I 0.2 (**I**) and phosphate buffer of pH 7.7 and I 0.2 (**II**); current 8 mA. Temperature was maintained at 23, 14, 12, 17, 0.5, 16, 23 and 17°C and exposure conducted 80, 60, 45, 45, 110, 60, 30 and 30 minutes after starting current in the cases of **I***a*, **I***b*, **I***c*, **I***d*, **II***a*, **II***b*, **II***c* and **II***d* respectively. Mobility:

I*a* $\dfrac{-0.6\times0.007305\times0.3}{4800\times0.008}=-3.42\ \mathrm{cm^2/volt/sec}\times10^{-5}$

I*b* $\dfrac{-0.25\times0.005565\times0.3}{3600\times0.008}=-1.45$ "

I*c* $\dfrac{-0.2\times0.005476\times0.3}{2700\times0.008}=-1.52$ "

I*d* $\dfrac{-0.3\times0.006465\times0.3}{2700\times0.008}=-2.69$ "

II*a* $\dfrac{-0.7\times0.005624\times0.3}{6600\times0.008}=-2.24$ "

II*b* $\dfrac{-0.4\times0.007229\times0.3}{3600\times0.008}=-3.01$ "

II*c* $\dfrac{-0.6\times0.012362\times0.3}{1800\times0.008}=-15.45$ "

II*d* $\dfrac{-0.4\times0.008702\times0.3}{1800\times0.008}=-7.25$ "

butylacetate-acetic acid-ethanol-water (3:2:1:1 by volume) according to Masamune and Yosizawa[8] (Fig. 2A and 2B).

When developed with the former solvent, the chromatograms of Glucidamin

TABLE I

Group Potencies of the Glucidamins

Anti-OS: an anti-OS saliva chicken serum. Goat: a normal anti-O goat serum. −: no hemagglutination, +: slight hemaggl., ++: marked hemaggl., ##: strong hemaggl.

Glucidamin	Red cells	Serum	Dilution of the substances (1:)							
			10^2	2×10^2	4×10^2	8×10^2	1.6×10^3	3.2×10^3	6.4×10^3	1.28×10^4
A	A	α	−	−	−	−	−	−	+	+
	B	β	++	##	##	##	##	##	##	##
	O	Anti-OS	++	++	++	++	##	##	##	##
	O	Goat	++	++	##	##	##	##	##	##
O	A	α	−	−	−	+	++	++	##	##
	B	β	−	−	−	+	++	++	##	##
	O	Anti-OS	−	−	−	−	+	+	++	++
	O	Goat	−	−	−	+	++	##	##	##

TABLE II

Test-tube Tests of the Preparations

Test	Preparation	
	Glucidamin A and O	Mucopolysaccharide I and II
Biuret	++	+*
Molisch	++	##
Bial-Mejbaum	−	+†
Neuberg-Saneyoshi	−	±
Hydrolyzable sulfur	−	−
Osaki-Turumi		
Indirect	±	+
Direct	+	++
Phosphorus	−	−

* Violet-red coloration. † A purple blue dye which passes into amylalcohol was produced.

A and O showed spots of galactosamine (weak), glucosamine, galactose, mannose and xylose, lyxose and/or L-fucose, and those of Mucopolysaccharide I and II spots of galactosamine (weak), glucosamine, galactose, glucose (weak), mannose and xylose, lyxose and/or L-fucose. And when irrigated with the latter solvent, the chromatograms of the glucidamins showed spots of galactosamine and/or glucosamine, galactose and/or glucose, mannose and L-fucose, and those of the mucopolysaccharides spots of galactosamine and/or glucosamine, of galactose and/or glucose, of mannose, xylose and/or lyxose (weak) and of L-fucose. In other words, Mucoprotein A and O proved to contain the both hexosamines, galactose, mannose and L-fucose, and Mucopolysaccharide I and II glucose and

Fig. 2A

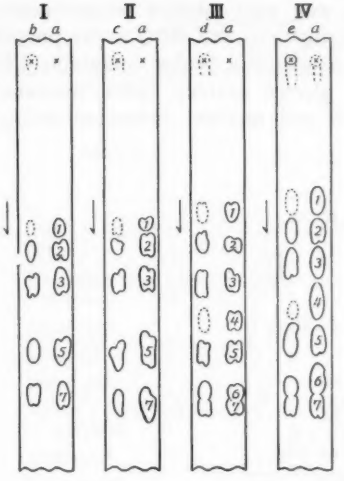

Fig. 2B

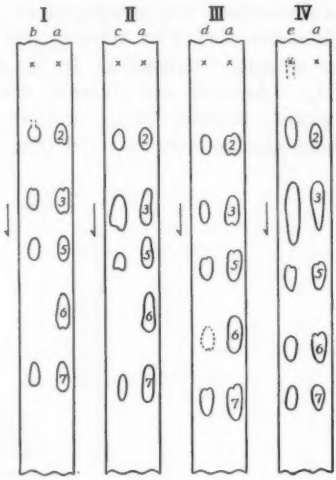

Fig. 2A. Sugar chromatograms (1/6×) of Glucidamin A (**I**) and O (**II**) and Mucopolysaccharide I (**III**) and II (**IV**) developed with Solvent butanol-pyridine-water mixture (5:3:2 by volume). Temperature of irrigation 23°±1°C. Duration of irrigation 25.5 hrs. (**I** and **III**) and 24 hrs. (**II** and **IV**). Indicator aniline hydrogen phthalate reagent of Partridge. Reference runs were carried out, applying 0.02 cc. of standard solutions which were 2% with respect to individual sugars.

a: reference run. *b*: Glucidamin A. *c*: Glucidamin O. *d*: Mucopolysaccharide I. *e*: Mucopolysaccharide II. *1*: galactosamine; *2*: glucosamine; *3*: galactose; *4*: glucose; *5*: mannose; *6*: xylose; *7*: L-fucose.

Fig. 2B. Sugar chromatograms (1/6×) of Glucidamin A (**I**) and O (**II**) and Mucopolysaccharide I (**III**) and II (**IV**) developed with Solvent *n*-butylacetate-acetic acid-ethanol-water mixture (3:2:1:1 by volume). Temperature and duration of test were 25°±1°C and 24 hrs. respectively. Indicator the same as in Fig. 2A. The standard solution was 1% with respect to individual sugars, and 0.02 cc. portions of it were applied.

a: reference run. *b*: Glucidamin A. *c*: Glucidamin O. *d*: Mucopolysaccharide I. *e*: Mucopolysaccharide II. Numbers 2–3 and 5–7 mean the same as in Fig. 2A. Glucose overlaps here with galactose.

xylose and/or lyxose in addition to those sugars.

Amino acids. 30 mg. of the mucopolysaccharide from lungs of Group A persons was heated with 3 cc. of 6 *N* HCl in a sealed tube at 100°C for 24 hours, and the hydrolysate was submitted to evaporation in a vacuum desiccator containing solid caustic soda at 35–36°C. The thick condensate was further evaporated with water repeatedly in a similar manner to expel off the hydrochloric acid as far as possible and finally stood till dryness in the desiccator. The final residue was taken up in 0.3 cc. of water, and 0.03 cc. of the

centrifuged supernatant was sampled on a paper square. A two-dimensional chromatogram was developed in the usual way with solvents butanol-acetic acid-water (4:1:2 by volume) (for primary irrigation) and 80% (w/w) phenol (for secondary irrigation; In the cylinder was placed a beaker containing 1% NH_3). Aspartic and glutamic acids, serine, glycine, alanine, lysine, histidine, arginine, proline, valine and/or methionine and leucine, isoleucine and/or phenylalanine were detected (Fig. 3).

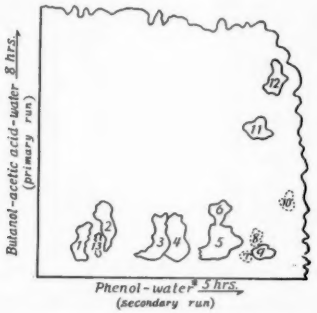

Fig. 3. Amino acid chromatogram (1/6×) of Mucopolysaccharide I. Temperature of test 23°±1°C. Indicator 0.1% ninhydrin in butanol saturated with water.

1: aspartic acid; *2*: glutamic acid; *3*: serine; *4*: glycine; *5*: hexosamine; *6*: alanine; *7*: lysine; *8*: histidine; *9*: arginine; *10*: proline; *11*: valine and/or methionine; *12*: leucine, isoleucine and/or phenylalanine; *13*: an unknown spot.

* Irrigation effected in an NH_3-containing atmosphere (See the text).

Quantitative analysis. The analytical figures are embodied in Table III. The contents of sugars differ not only in per cent but also in equivalent ratio between the glucidamins belonging to different blood groups, but do not at all between the mucopolysaccharide from lungs of Group A persons and that from lungs of Group O persons. Besides their similarity in composition, those mucopolysaccharide preparations have practically the same optical rotations as will be described below and are both group inactive. Therefore they are most probably identical with each other.

Optical rotation.

Glucidamin A, $[\alpha]_D^{25}$ (0.01 *N* NaOH) $= \dfrac{-0.122 \times 1}{0.0079 \times 1} = -15.4°$

Glucidamin O, $[\alpha]_D^{25}$ (0.01 *N* NaOH) $= \dfrac{-0.600 \times 1}{0.0114 \times 1} = -52.8°$

Mucopolysaccharide I, $[\alpha]_D^{20}$ (water) $= \dfrac{-0.1595 \times 1}{0.0099 \times 1} = -16.1°$

Mucopolysaccharide II, $[\alpha]_D^{20}$ (water) $= \dfrac{-0.1665 \times 1}{0.0102 \times 1} = -16.3°$

TABLE III

Compositions of Glucidamin A and O and Mucopolysaccharide I and II

Analysis	In per cent				In equivalents per equivalent weight			
	Glucidamin		Mucopolysaccharide		Glucidamin		Mucopolysaccharide	
	A	O	I	II	A	O	I	II
N*	10.4	8.4	5.2	5.0	11.1	8.9	3.3	3.0
Hexosamines as glucosamine†	12.1	13.0	20.5	21.6	1.0	1.0	1.0	1.0
Galactose‡	7.6	14.0	11.8	13.0	0.6	1.2	0.6	0.6
Glucose‡			4.6	3.3			0.2	0.2
Mannose‡	9.3	1.8	11.9	12.4	0.7	0.1	0.6	0.6
Xylose and/or lyxose as xylose§			4.6	4.4			0.3	0.2
L-Fucose//	2.0	3.8	2.9	4.1	0.2	0.3	0.2	0.2
Sialic acid¶	13.5	8.6	9.4	9.9	0.6	0.4	0.3	0.3
Ash**	0.4	0.4	7.1	3.4				

* Micro Kjeldahl. † A minor modification of Blix method (Masamune and Yosizawa[9]). ‡ Masamune and Sakamoto.[10] § Masamune and Sakamoto.[11] // A modification of Dische and Shettles method.[12] ¶ Werner and Odin's direct Ehrlich method[13]; Calcd. as $C_{11}H_{19}NO_9$.[14] ** Pregl method without use of H_2SO_4.

SUMMARY

1. A glucidamin- and a mucopolysaccharide-preparation were obtained from lungs of Group A persons and from those of Group O persons, and examined regarding their properties physical and chemical.

2. The glucidamin from Group A lungs is Group A active, but that from Group O lungs slightly inhibited agglutination of Group O, A and B erythrocytes by the respective antibodies, which might be ascribed to group interconversion during preparation. The mucopolysaccharide from lungs of Group A persons and that from lungs of Group O persons are both group inactive.

3. The glucidamins differ from each other in quantitative composition as well as in optical rotation, whereas the mucopolysaccharide preparations have resembling compositions and rotations, indicating their identity. The sugar components of the glucidamins are galactosamine, glucosamine, galactose, mannose and L-fucose. The mucopolysaccharide contains glucose and xylose and/or lyxose besides the sugars in the glucidamins.

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Studies of High-molecular Placental Components in Connection with Pregnancy Toxemia

Fifth Paper:

On Placental Itinsulfuric Acids*

By

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From fresh placentas were prepared itinsulfuric acids which looked to be the same in composition as cartilage chondroitinsulfuric acid. Because of little yields, however, only one of the products could be submitted to biological assays. The results indicated it to have no connection with pregnancy toxemia contrary to the findings by Kanno *et al.*[1] on a crude mixture of the itinsulfuric acids which had been obtained from placentas after boiling with alcohol.

It is particularly to mention that the present work disclosed the presence of at least three itinsulfuric acids in human placenta.

Experimental

Preparation of Itinsulfuric Acids from Placentas

Prep. I. 1st stage. 30 fresh human placentas freed from blood vessels and adhering membranes were ground and united. The brei that weighed 9513 g. was added to with 1.5 volumes of 0.14 *M* NaCl containing 0.01 *M* sodium citrate, stirred up at 0°C in a freeze mixture for 24 hours and centrifuged. The centrifugate was extracted with decreasing volumes of the saline mixture twice more. The red turbid solutions were combined together, acidified to pH 4.2 and placed in an ice-box overnight. The voluminous brown precipitate was centrifuged off, added to with 1.5 *l.* of 3 *M* NaCl, and after adjusting to pH 7.0 with 10% NaOH, agitated for 12 hours (at 0°C) in a freeze mixture, followed by standing in the ice-box for two days. It was acidified to pH 4.2, again placed in the ice-box for several hours and filtered. The bulk on the funnel was further exhausted twice, employing 50 cc. of 3 *M* NaCl per placenta as above. The filtrates were united, and 3 volumes of 91% alcohol were added. After standing overnight,

* 204th report of Masamune and co-workers' "Biochemical Studies on Carbohydrates."

the deposit was centrifuged off. It was taken up in 10 cc. of water per placenta, and 1% NaOH was added to pH 6.8. The insoluble part was rejected. The clear solution was precipitated with 3 volumes of ice-chilled abs. alcohol and a few drops of abs. alcohol saturated with crystalline sodium acetate. The deposit here was subjected to redissolution and reprecipitation twice and dried in a vacuum-desiccator containing $CaCl_2$. 4.09 g. was the yield. Biuret +, Molisch ++, Goldschmiedt +, Neuberg-Saneyoshi ++, Bial ++, Dische +.

2nd stage. It was dissolved in 250 cc. of 3 *M* NaCl by means of a homogenizer, adding 10% NaOH to pH 6.8 from time to time, and after standing in the ice-box for 1 day, deproteinized with about 30 changes of an amylalcohol-chloroform (1:4) mixture according to Sevag. The watery solution was added to with 3 volumes of chilled abs. alcohol, stood for 6 hours in a freeze mixture and centrifuged. The centrifugate was taken up in 150 cc. of water, dialyzed against running water for 3 days (Temperature of the running water 5°C) and distilled at 20°C to about 150 cc. The precipitate was filtred off and rejected. The filtrate was chilled to 0°C, and 10% HCl was added to pH 1.8. The white flocculent precipitate was centrifuged off, reprecipitated from water three times at the same pH as above, and set aside as a material for isolation of the pentose-nucleic acid—Crude PNA I (See the forthcoming paper[2]). The mother fluids here (230 cc. in total) were combined, neutralized to pH 6.8 and distilled to about 100 cc. It was liberated from the still remaining PNA by careful addition of 20% $CuCl_2$, and then precipitated with 1.5 volumes of 99% alcohol. The bluish deposit was freed from copper by reprecipitations through pouring its solution in 20 cc. of water into 200 cc. of alcohol containing 0.05 *M* HCl. The almost decolorized substance (gelatinous) was washed thoroughly with HCl-containing alcohol and plain alcohol and dried in vacuo over $CaCl_2$. 292 mg. was the dry powder given—Crude Itinsulfuric Acids.

3rd stage. It was dissolved in 20 cc. of water. The solution was adjusted to pH 6.8 with 1% NaOH, and 5 cc. of 20% barium acetate (crystalline) and 10 cc. of chilled 99% alcohol were added in turn. The deposit here was reprecipitated 10 times from 20 cc. of water as above and dried also as above.

4th stage. It was kneaded with 1 cc. of water and agitated with 49 cc. of 90.8% (w/v) phenol. The insoluble part was submitted to many repetitions of the same treatment. A non-colored jelly thus given was washed thoroughly with ether to eliminate phenol and dried. It was then dissolved in 40 cc. of water, and a small amount of N_2SO_4 was added to carry down barium. Filtered. The clear filtrate was dialyzed for 3 days and precipitated with 5 volumes of chilled 99% alcohol containing some crystalline sodium acetate. The precipitate was separated, washed with alcohol and ether and dried. 255 mg. of a white powder was obtained. Biuret −, Molisch +, Goldschmiedt ++, Neuberg-Saneyoshi ++.

5th stage. The product was dissolved in 4 cc. of acetate buffer pH 4.6, I 0.1 and fractioned by the electrophoretic sedimentation method of Masamune *et al.*[3] (300 V, 10 mA, 5 hours). The refractive layer in compartment j_2 which contained the moving ingredients was precipitated with 5 volumes of 99%

alcohol, washed with alcohol and ether and dried. It weighed 226 mg. Biuret —, Molisch ±, Goldschmiedt ++, Neuberg-Saneyoshi ++, Dische —, Bial +.

6th stage. It was dissolved in 20 cc. of water and acidulated with 10% acetic acid to pH 5.0, and a 10% solution of $(CH_3COO)_2Pb{\cdot}3H_2O$ was added in drops. The small white deposit was discarded after centrifugation. The supernatant was precipitated with 1/2 the volume of 99% alcohol, stirred up in 15 cc. of water and added to with 10 cc. of 2.5% ammonium acetate, whereby the substance dissolved perfectly. This solution was added to with 10 cc. of the same lead acetate solution as above and stood for a while. A tiny precipitate that occurred was rejected after centrifugation and the supernatant was precipitated with alcohol as above. Dissolution and precipitation was processed twice more, and the last precipitate was dissolved in 5 cc. of 2.5% ammonium acetate and electrodialyzed after dilution to 50 cc. with water. The inner fluid which was free from lead was distilled in vacuo to 5 cc., precipitated with an alcohol-ether (1:1) mixture and dried in vacuo over $CaCl_2$ to a constant weight. A part of it was submitted to electrophoresis in acetate buffer (See below) and thereafter recovered by precipitation with alcohol to unite with the balance. The substances put together were dissolved in 10 cc. of water, and 150 cc. of 99% alcohol and a small volume of 99% alcohol saturated with sodium acetate (crystalline) were added. The deposit was centrifuged off after standing overnight in the ice-box, washed exhaustively with 99% alcohol and ether and dried in vacuo over $CaCl_2$. 95 mg. of a white powder (sodium itinsulfates) was given.

Preps. IIA, IIB and IIC. 1st stage. The tissue brei obtained as above from 116 placentas weighed 32,402 g. It was extracted with 2 and 1.5 volumes of 0.4 *M* NaCl by agitation at 0°C in a freeze mixture. The solutions combined were acidified to pH 4.2, and the deposit hereby was separated after standing in an ice-box overnight and again dissolved in 100 cc. of 3 *M* NaCl per placenta, shifting the pH to 7.0 by means of 10% NaOH. The solution here was placed for 2 days in an ice-box, and after further agitation for 1 day under cooling on ice, acidified to pH 4.2 with 10% HCl, followed by standing in the ice-box overnight. Filtered under cooling. The bulk on the funnel was treated in a smiliar manner over again. The filtrates (biuret +, Molisch +, Bial +, Neuberg-Saneyoshi ++, Goldschmiedt +, Dische +) were precipitated by addition of 3 volumes of chilled 91% alcohol. Centrifuged. The yellowish centrifugate was dissolved in 1.16 *l.* of water and precipitated with 3 volumes of chilled abs. alcohol containing some sodium acetate (crystalline). The non-colored deposit thus given was separated after maintaining at 0°C in a freeze mixture for several hours and dried in vacuo over $CaCl_2$—A mixture of itinsulfuric acids and PNA. Yield 16.8 g.

2nd stage. It was pulverized and dissolved in 500 cc. of chilled 3 *M* NaCl by the aid of a homogenizer, adding 10% NaOH to pH 6.8 from time to time, and stood for 48 hours in an ice-box with occasional agitation to make nucleic and itinsulfuric acids dissociate from proteins. Centrifuged. The tiny insoluble part was biuret ++, Molisch +, Goldschmiedt ±. The supernatant

was deproteinized at 0–5°C by the procedure of Sevag with more than 50 changes of an amylalcohol-chloroform mixture in the volume proportion of 1:4. The solution which was uncolored and transparent was acidified to pH 4.2 with 20% acetic acid, and 2 volumes of chilled 99% alcohol were added. After standing at 0°C for 12 hours, the flocculent precipitate was separated in a refrigerated centrifuge, dissolved in 150 cc. of water and dialyzed. The solution was then distilled at room temperature (20°C) to 50 cc., acidified to pH 1.6 with 10% HCl and stood at 0°C for 10 hours, followed by centrifugation at a temperature lower than 0°C. The centrifugate (Crude PNA II) was washed with very dilute HCl of pH 1.6 at 0°C and submitted to isolation of PNA (See the forthcoming paper[2]).

3rd stage. The centrifuged supernatant and washing (130 cc. in total) were added to with 10% NaOH to pH 6.8, distilled at 20°C in vacuo to about 60 cc. and liberated from most of the remaining nucleic acids by shifting its pH to 1.6 as above. The solution was again neutralized to pH 6.8 and added to with 20 cc. of a 20% solution of crystalline barium acetate, which had been adjusted to pH 6.8 with acetic acid, and the mixture was stood at 0°C for 1 day. A trace of a white precipitate that occurred was discarded after centrifugation, and the supernatant was dialyzed for 3 days, added to with some sodium sulfate and filtered. The filtrate here was dialyzed, concentrated similar to above to 30 cc., and after addition of 1% NaOH to pH 7.8, further added to with 10 cc. of 20% barium acetate (crystalline) and with the saturated baryta to pH 7.8, followed by standing in the ice-box overnight. The precipitate was removed, and the clear mother fluid was dialyzed. Addition of sodium sulfate, filtration, dialysis and condensation to 30 cc. was repeated. And, the condensate was acidified to pH 5.0 with acetic acid and cooled to 0°C, and 10% lead acetate (crystalline) was added in drops until no more precipitate occurred. After standing for 5 hours in a freeze mixture, the deposit (gelatinous) was separated in the refrigerated centrifuge—Fr. 1. The supernatant was precipitated with 2 volumes of chilled 99% alcohol. The deposit was taken up in 30 cc. of 4% ammonium sulfate, dialyzed and freed from lead by means of 10% Na_2CO_3. The solution was dialyzed again and passed through columns (1×31.5 cm.) of Amberlite IRA-410 and of Amberlite IR-120 in succession. The effluent and washing were distilled in vacuo at about 20°C to 75 cc., adjusted to pH 6.8 with 10% NaOH and added to with 25 cc. of 20% barium acetate (crystalline). Of the solution a part was chilled to 0°C, and alcohol similarly chilled was added in small portions. Hereby the precipitate increased not continuously but occurred at alcohol concentration ranges 8.0–11.8% and 14.0–16.6% with maxima at about 10% and 15–16% respectively. Therefore, the whole balance was fractioned under the same conditions. A comparatively small precipitate on addition of alcohol to 11.8% was separated after centrifugation (Fr. 2) and a larger precipitate appearing from the centrifuged supernatant on increasing the alcohol concentration to 16.6% was also separated (Fr. 3). The mother fluid here gave trifling precipitates on addition of additional alcohol to 40% and furtheron to 70% (Fr. 4 and 5).

Purification of Fr. 1. The non-colored, gelatinous precipitate was washed with a 10% lead acetate (crystalline) solution (pH 6.0) several times and suspended in 40 cc. of water, and 10% Na_2CO_3 was added until no more occurrence of turbidity. Filtered. The filtrate was dialyzed, treated with Amberlite IRA-410 and IR-120 and distilled at room temperature to a syrup which was precipitated with an alcohol-ether mixture (4 : 1 by volume), washed with ether and dried in vacuo over $CaCl_2$. A white powder weighing 55 mg. was yielded. A part of it was submitted to electrophoresis and recovered by precipitation with alcohol to unite with the balance. The combined substances were taken up in 10 cc. of water, precipitated with an ice-chilled mixture of 30 cc. of plain 99% alcohol and 3 cc. of 99% alcohol saturated with crystalline sodium acetate. After standing in an ice-box overnight, the deposit was centrifuged off and washed with alcohol and ether and dried in vacuo over $CaCl_2$ to a constant weight—**Prep. IIA**. Yield 59 mg.

Purification of Fr. 2. The crude fraction was dissolved in 30 cc. of water, and after addition of 10 cc. of 20% barium acetate (crystalline), added to with 99% alcohol to 11.8%, followed by standing at 0°C for 6 hours. The flocculent precipitate was separated at 0°C in the refrigerated centrifuge. The centrifugate was treated in a similar manner many times. The last centrifugate was taken up in 40 cc. of water, freed from barium with some Na_2SO_4, and after dialysis and subsequent passing through the resins above, vacuum-distilled at room temperature to about 15 cc. It was next added to with 5 cc. of 10% lead acetate (crystalline) and stood in an ice-box (0°C) for 24 hours. An occurring tiny white precipitate was centrifuged off and rejected. The supernatant was precipitated with two volumes of 99% alcohol and dried in a desiccator. It was again taken up in 30 cc. of water. A very small part was insoluble. The centrifuged supernatant here was freed from lead, condensed, precipitated, washed with ether and dried, as described in " Purification of Fr. 1 ", giving 48 mg. of a white powder. It was further changed into sodium salt as above—**Prep. IIB.** Yield 51 mg.

Purification of Fr. 3. The crude fraction was taken up in 30 cc. of water, and after addition of 10 cc. of 20% barium acetate (crystalline), fractioned with alcohol. The precipitate occurring on addition of alcohol to 11.8% was separated after standing at 0°C for 6 hours and discarded, and the precipitate from the mother fluid on increasing alcohol concentration to 16.6% was recovered. This fractionation procedure was carried out 9 times more. The last centrifugate was dissolved in 40 cc. of water, treated with Na_2SO_4 and the resins, and condensed to 30 cc. It was further submitted repeatedly to the treatment with lead acetate and following operations above. The free itinsulfuric acid obtained weighed 133 mg. when dry. This was changed into sodium salt also as above. 142 mg. were yielded—**Prep. IIC.**

Fr. 4 and 5 were also purified by alcoholic fractionation in the presence of barium acetate. Qualitatively tested, they proved to be Molisch positive mucopolysaccharides.

Physical and Chemical Properties

Test-tube tests. All the preparations gave the same results: biuret, Molisch, Dische (for deoxypentose) and phosphorus −; Goldschmiedt and Neuberg-Saneyoshi (for hexuronic acid), Bial (for pentose) and hydrolyzable sulfur +.

Electrophoresis. The substances before changing into sodium salts demon-

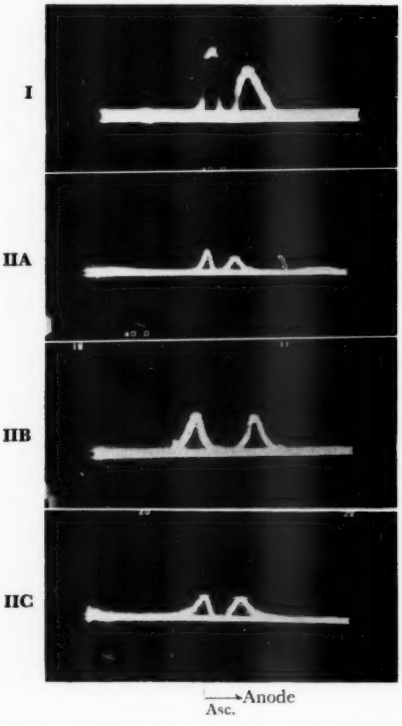

Fig. 1. Electrophoretic patterns (4/5×) of 1% solutions in acetate buffer pH 4.6, I 0.4 of Prep. I (**I**), IIA (**IIA**), IIB (**IIB**) and IIC (**IIC**); current 10 mA; temperature of test 18°C in Prep. I and 10°C in the other preparations. Exposed 55, 56, 50 and 50 minutes after starting current respectively in the preparations as arranged as above. Mobility:

Prep. I (18°C), $\frac{-1.6\times0.009169\times0.3}{3300\times0.01}=-13.33$ cm²/volt/sec × 10^{-5}

Prep. IIA (10°C), $\frac{-1.65\times0.008597\times0.3}{3360\times0.01}=-12.66$ "

Prep. IIB (10°C), $\frac{-1.40\times0.008965\times0.3}{3000\times0.01}=-12.55$ "

Prep. IIC (10°C), $\frac{-1.50\times0.008965\times0.3}{3000\times0.01}=-13.44$ "

strated all only one component after electrophoresis at pH 4.6. See Fig. 1.

Paper partition chromatography of the sugar components. 5 mg. of Prep. I, IIB and IIC and 8 mg. of Prep. IIA were sealed each with 1 cc. of 1 *N* HCl in a tube and heated at 100°C for 11 hours. The hydrolysates were freed from the hydrochloric acid by evaporation in the manner described by Masamune *et al.*[4] and finally dried. The residues were dissolved separately in 0.3 cc. of water. 0.04 cc. (in the case of Prep. I) and 0.03 cc. (in the other cases) portions of the solutions were sampled for the chromatography of hexosamine and that of hexuronic acid. As shown in Fig. 2–5, spots were given at the positions corresponding to galactosamine and glucuronic acid in any of the preparations.

Fig. 2

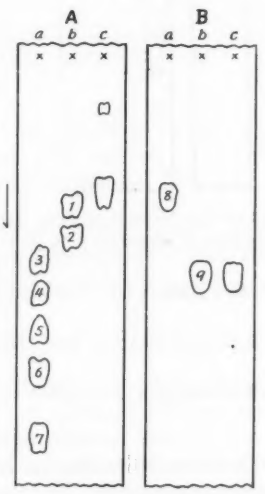

Fig. 3

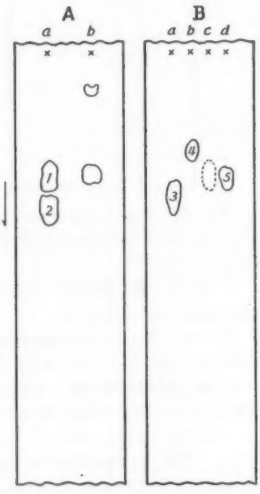

Fig. 2. Hexosamine (**A**) and hexuronic acid (**B**) chromatograms (1/6×) of the hydrolysate of Prep. I developed by descending irrigation with Solvent butanol-pyridine-water (5:3:2 by volume). Duration of irrigation: **A** 24 hrs.; **B** 84 hrs. Temperature of irrigation 22°C. Indicator aniline hydrogen phthalate reagent of Partridge. Standard solutions were 1% with respect to individual sugars, and a 0.025 cc. portion of them were spotted.

a, *b*: reference run. *c*: main run. *1* galactosamine, *2* glucosamine, *3* galactose, *4* glucose, *5* mannose, *6* L-fucose, *7* rhamnose, *8* galacturonic acid, *9* glucuronic acid.

Fig. 3. Paper chromatograms (1/6×) of the hydrolysate of Prep. IIA developed and indicated as in Fig. 2. Duration of irrigation: **A** 24 hrs., **B** 72 hrs. Temperature of irrigation 23°±1°C.

A*a*, **B***a*, **B***b*, **B***d*: reference run. **A***b*, **B***c*: main run. *1* galactosamine, *2* glucosamine, *3* mannuronic acid, *4* galacturonic acid, *5* glucuronic acid.

Fig. 4

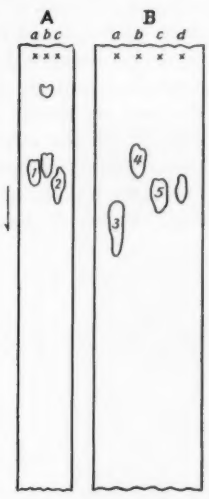

Fig. 5

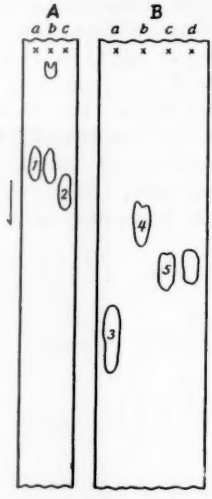

Fig. 4. Paper chromatograms (1/6×) of the hydrolysate of Prep. IIB developed and indicated as in Fig. 3. Temperature of irrigation: **A** 22°C, **B** 24°±1°C.

A*a*, **A***c*, **B***a*, **B***b*, **B***c*: reference run. **A***b*, **B***d*: main run. *1*~*5* signify the same as in Fig. 3.

Fig. 5. Paper chromatograms (1/6×) of the hydrolysate of Prep. IIC developed and indicated as in Fig. 4.

A*a*, **A***c*, **B***a*, **B***b*, **B***c*: reference run. **A***b*, **B***d*: main run. *1*~*5* signify the same as in Fig. 3 and 4.

Analysis. The preparations (sodium salts) gave analytical figures embodied in Table I, which indicates that all the substances are composed of equimolecular acetylgalactosamine, glucuronic acid and sulfuric acid.

Optical rotations in water:

Prep. I, $[\alpha]_D^{23} = \frac{-0.314 \times 1}{0.0157 \times 1} = -20.0°$

IIA, $[\alpha]_D^{14} = \frac{-0.649 \times 1}{0.0103 \times 1} = -63.0°$

IIB, $[\alpha]_D^{23} = \frac{-0.218 \times 1}{0.0104 \times 1} = -21.0°$

IIC, $[\alpha]_D^{14} = \frac{-0.195 \times 1}{0.0109 \times 1} = -17.9°$

Preps. IIA–IIC all resemble one another in electrophoretic mobility in the acetate buffer as examined as free acids, but with regard to the optical rotation and lead-precipitability, Prep. IIA differs from Prep. IIB and IIC which are similar on their side, and alcohol-precipitability of the barium salts discriminates

TABLE I

Composition of the Placental Itinsulfuric Acids

Analysis	In per cent				In equivalents per equivalent weight			
	Prep. I	IIA	IIB	IIC	Prep. I	IIA	IIB	IIC
N*	2.67	2.41	2.66	2.76	1.00	0.88	1.02	1.05
Galactosamine†	33.95	34.87	33.55	33.64	1.0	1.0	1.0	1.0
Glucuronic acid								
Thionalide method‡	35.77	34.09	36.39	36.21				
Thioglycolic acid method§	34.00	35.45	35.60	35.25				
Mean	34.89	34.77	36.00	35.73	0.95	0.92	0.99	0.98
Acetyl//		8.06		9.24		0.96		1.14
Hydrolyzable sulfur¶	5.47	7.39	5.44	5.55	0.90	1.18	0.91	0.92
Ash**	26.40	26.55		26.35				

* Micro Kjeldahl. † Blix. ‡ Masamune, Sakamoto and Aizawa.[5] § Masamune and Aizawa.[6] // Suzuki's modification of Friedrich, Rapoport and Sternberg method.[7] ¶ Suzuki.[7] ** Pregl.

Prep. IIB and IIC from each other. Prep. I must be a mixture of the salts of itinsulfuric acids identical with Prep. IIB and IIC, because the lead-precipitate which contained the itinsulfuric acid corresponding to Prep. IIA was removed.

Biological Properties

The balance of Prep. IIC was used for this examination, a part of which Dr. H. Kawakami carried out in the Department of Obstetrics and Gynecology, Fukushima Medical School and will publish elsewhere[8] in detail. The findings will be briefly described in the following.

1) Effect on liver and kidney of pregnant rabbits (Experiment by Kawakami). 5 mg. per kg. body weight of the substance was dissolved in 200 volumes of physiological saline and injected to pregnant rabbits intravenously (auricular vein), and the animals were killed after 24 hours. The histological findings in liver and kidney are shown in Table II.

2) Effect on blood pressure of pregnant rabbits. Pregnant rabbits underwent no change of blood pressure in carotic artery when injected with the substance in the same dose and manner as in 1). See Table III.

3) Effect on time of pomphus absorption in pregnant rabbits. 0.1 cc. portions of physiological saline were injected subcutaneously on the inner side of the pinna of pregnant rabbits 10 minutes before and after the substance was injected as above, and the time of absorbing the pomphus was determined by inspection and palpation. As shown in Table IV, pomphus absorption was neither quickened nor delayed by the itinsulfuric acid, indicating that the substance has no edema-inducing potency.

TABLE II

Pathological Changes in Liver and Kidney of Pregnant Rabbits after Injection of Prep. IIC

Symbol − with respect to histological findings signifies no change.

Rabbit No.	Rabbit Wt.(g.)	Days of pregnancy	Time of killing animal after inj. (hrs.)	Liver							Kidney		Premature delivery	Aggravation of general status after injection†
				Necrosis	Necrobiosis	Cloudy swelling	Degeneration	Stasis of blood stream	Thrombosis	Change of Glisson capsule*	Nephrosis	Glomerulonephrosis		
136	3000	24	24	−	−	−	−	−	−	−	−	−	−	+
137	3000	22	24	−	−	−	−	−	−	−	−	−	−	±

* Cellular infiltration and swelling of the sheath. † ± : faint aggravation ; + slight aggravation.

TABLE III

Blood Pressure in Carotic Artery of Pregnant Rabbits after Injection of Prep. IIC

Rabbit No.	Rabbit Wt.(g.)	Days of pregnancy	Blood pressure (mm. Hg)					
			Before injection	Time after injection (min.)				
				0.5	1	3	5	7
138	3000	24	102	102	107	104	99	102
139	3000	22	108	108	108	108	104	104

TABLE IV

Effect of Prep. IIC on Time of Absorbing Pomphus Produced with Physiological Saline

Rabbit No.	Rabbit Wt.(g.)	Days of pregnancy	Time of pomphus absorption (min.)	
			Before injection of Prep. IIC	After injection of Prep. IIC
140	3000	22	6	5
141	3000	24	7	6

4) One single intravenous injection of the substance in the above dose entailed no albuminuria in pregnant rabbits.

5) The substance showed no precipitin reaction with the sera not only of the non-pregnant and normal-pregnant women but also of the pregnancy-

toxemic. See Table V.

6) Skin reaction. The substance was taken up in 1500 volumes of physiological saline containing 0.5% phenol, and 0.1 cc. portions of the solution were injected intracutaneously on flexor side of forearm of non-pregnant, normal-pregnant, puerperal, pregnancy-toxemic and uterus cancer-suffering women. The average diameters of the rednesses were measured after 4 hours. No significant differences of reddened area were found among the different groups of individuals. See Fig. 6.

TABLE V

Precipitin Reaction of Prep. IIC with the Sera of Non-pregnant, Normal-pregnant and Pregnancy-toxemic Women

Symbol – signifies no occurrence of the reaction.

No. of individual	Dilution of Prep. IIC in physiol. saline (1:)				Symptoms of patients		
	10^2	2×10^2	3×10^2	4×10^2	Blood pressure (mm. Hg)	Albuminuria	Edema
Sera of non-pregnant women							
1	–	–	–	–			
2	–	–	–	–			
3	–	–	–	–			
4	–	–	–	–			
5	–	–	–	–			
Sera of normal-pregnant women							
6	–	–	–	–			
7	–	–	–	–			
8	–	–	–	–			
9	–	–	–	–			
10	–	–	–	–			
11	–	–	–	–			
Sera of pregnancy-toxemic women							
12	–	–	–	–	156–100	(++)	(+)
13	–	–	–	–	136– 90	(+++)	(+)
14	–	–	–	–	120– 90	(–)	(+)
15	–	–	–	–	158– 90	(+)	(++)
16	–	–	–	–	150– 90	(+)	(++)
17	–	–	–	–	160–100	(+)	(++)

SUMMARY

From fresh placentas were isolated three itinsulfuric acids most probably containing acetylgalactosamine and glucuronic acid as sugar components. One of them was proved to play no role in inducing pregnancy toxemia.

Through the Grant Committee for Scientific Researches the Ministry of

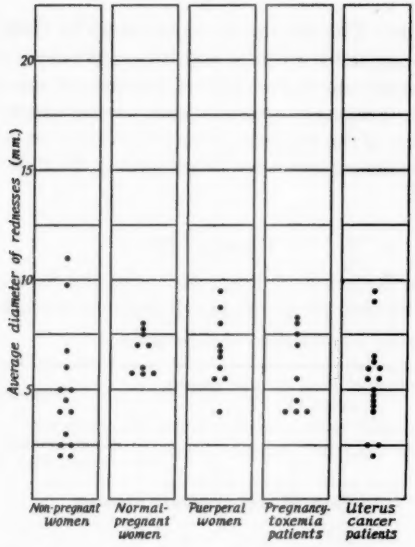

Fig. 6. Showing the results of skin reaction.

Education gave a grant in aid to us, which is gratefully acknowledged. H. Masamune.

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Dietary Hepatic Necrosis of Albino Rats Induced by Japanese Brewer's Yeast

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Massive hepatic necrosis has been observed in albino rats fed a low protein diet deficient in cystine, α-tocopherol (Vitamin E) and a third as yet unidentified substance called Factor 3. In Japan, Anbo[1] observed massive hepatic necrosis in rats receiving casein as the source of dietary protein. As yet, there are no reports concerning the use of Japanese brewer's yeast for producing experimental dietary hepatic necrosis. A following attempt was made to study dietary hepatic necrosis using Japanese brewer's yeast as the source of dietary protein.

Experimental

Material and Methods

Seventeen male albino rats of the Lister strain having an average body weight of 58 gm. were fed with a low protein diet, deficient in cystine, α-tocopherol and Factor 3. The composition of the diet was as follows: Japanese brewer's yeast 18%, sucrose 71%, lard 5%, cod liver oil 2% and Osborne-Mendel's salt mixture 4%. Vitamins were added to supply the following amounts daily: Thiamine 50 μg., riboflavin 50 μg., pyridoxine 20 μg., calcium pantothenate 100 μg. and nicotinamide 500 μg. The rats received 8 to 12 gm. of this diet per day. The room temperature during the five to nine weeks of the experiment ranged from 18° to 26°C. Histological examinations of the liver were done using hematoxylin-eosin, Sudan III and van-Gieson stains.

Results

One rat died from pneumonia. Of the remaining 16 rats, 8 died from massive hepatic necrosis. Their deaths occurred after receiving the special diet for 24 to 48 days (average: 33 days). Fig. 1 shows the body weight during the experiment for each of the rats. All rats gained weight while on the diet. An abrupt decrease in weight was observed shortly

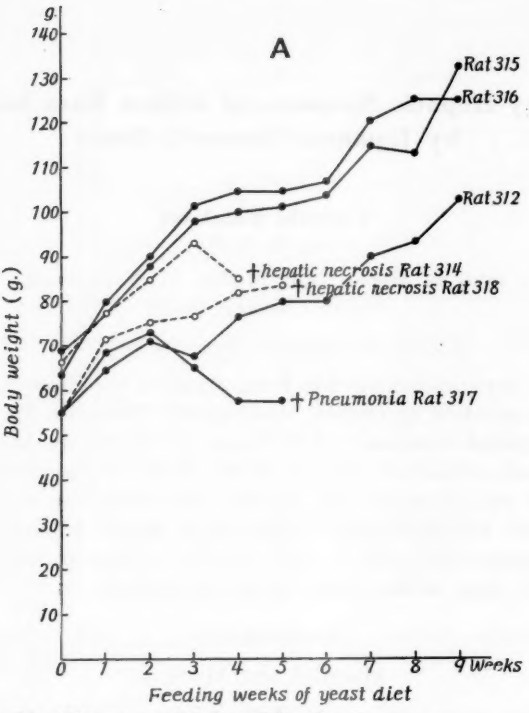

Fig. 1A. The curves of body weight of each rats.

before death in 3 of the 8 rats that died from hepatic necrosis. Other than this, they appeared healthy until the day of death. The 8 surviving rats appeared healthy and gained weight in a normal fashion, until they were killed, 39 to 69 days after starting on the special diet. Their livers were normal both macroscopically and microscopically. The liver of rats dying from hepatic necrosis were enlarged markedly as shown in Fig. 2. Average liver weight, expressed as percent of body weight, was 125% that of normal rats (6.9% for rats dying from hepatic necrosis; 5.5% for normal rats). The necrotic livers were soft and friable and presented a coarsely mottled appearance with yellowish-gray necrotic areas and reddish-brown hemorrhagic areas. Microscopically the chief change was massive necrosis of coagulative type with hemorrhage and congestion. Focal necrosis and hemorrhage of a central and midzonal distribution were seen in many lobules contiguous to areas of massive necrosis (Fig. 3). These necrotic and hemorrhagic areas were most frequently seen adjacent to Glisson's capsule. In the central area of the lobules,

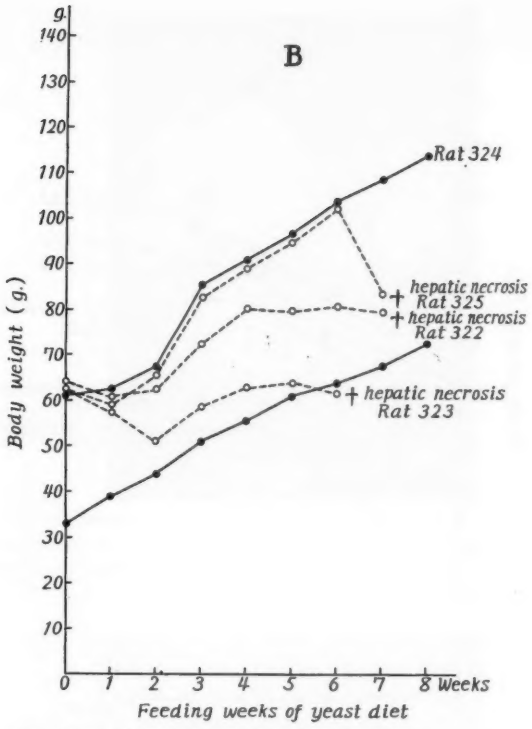

Fig. 1B. The curves of body weight of each rats.

liver cells maintained a normal contour but their cytoplasm was decreased and showed a finely granular turbidity. The remaining viable cells in the periphery of the lobules appeared the same as cells in the central zone, except that the former were better stained. Various nuclear changes were seen, including pyknosis, karyolysis and karyorrhexis. There was no significant inflammatory cell infiltration but cell infiltrations consisting of mainly mononuclear cells with some small round cells and neutrophilic leucocytes were occasionally observed. Hyperplasia of the bile ducts appeared in some of the portal tracts. Fibrosis was not observed in the van-Gieson stained sections. Fine granular fatty cysts were observed in the necrotic areas and in the remaining viable liver cells, and sometimes diffuse fatty infiltration was seen in the lobules and periportal areas in the section of Sudan III stain (Fig. 4). The lungs were normal macroscopically except for the rat which died from pneumonia. Histological changes were slight or absent in the kidneys. Changes when

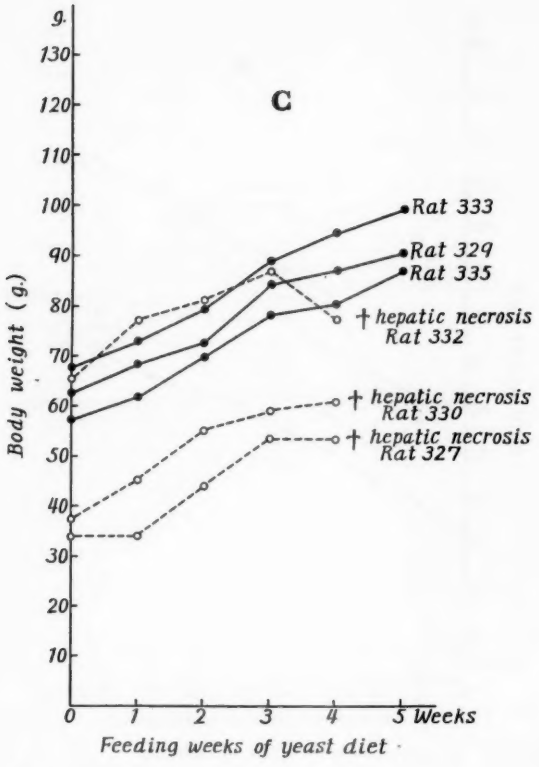

Fig. 1C. The curves of body weight of each rats.

present consisted of slight glomerular hyperemia and or slight degenerative changes in the tubules. Myocardial lesions were not observed.

Discussion

The pathology of dietary hepatic necrosis of the rat resembles acute yellow atrophy of the liver in man. The histological changes observed in the 8 rats dying from hepatic necrosis were the same as the " massive hepatic necrosis " of Himsworth[2] and Abell[3] and the " acute diffuse necrosis " of György.[4] In previous studies, the incidence of hepatic necrosis has been high (Abell, 98% after 38 days; Himsworth, 88% after 44 days; and MacLean,[5] 100% after 73 days). The relatively low incidence (50%) encountered in my studies. György, Lindan[6] and Schwarz[7] analyzed British baker's yeast and American brewer's yeast

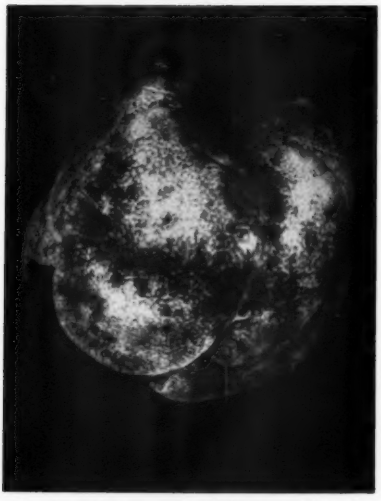

Fig. 2. Massive hepatic necrosis of the rat. The liver was enlarged markedly and soft and friable and presented a corsely mottled appearance with yellowish-gray necrotic areas and reddish-brown hemorrhagic areas. 2/5×.

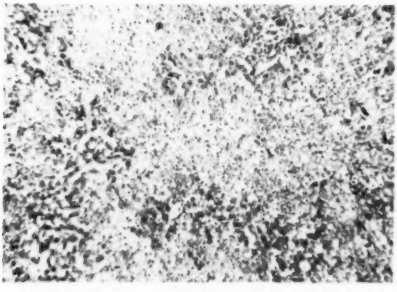

Fig. 3. Section of the liver, showing focal necrosis and hemorrhage of central and midzonal distribution in many lobules contiguous to areas of massive necrosis. Hematoxylin and eosin staining; 120×.

chemically but they found no differences in chemical composition, such as sulphur-containing amino acids (methionine and cystine) vitamin E and glutathion *etc.*, between the two kinds of yeast. Recently Schwarz stated that British brewer's yeast, which lacks Factor 3 and causes a high incidence of dietary hepatic necrosis under suitable experimental conditions, and American brewer's yeast and casein which contains Factor 3, and therefore does not cause dietary hepatic necrosis, for although it

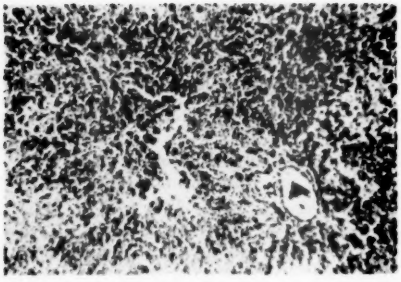

Fig. 4. Section of the liver, showing diffuse fatty infiltration in the lobules and periportal areas. Frozen section stained with Sudan III ; 120×.

lacks cystine and α-tocopherol. Japanese brewer's yeast may be relatively suitable for producing dietary hepatic necrosis, for it lacks these three dietary factors. Himsworth and Abell observed partial necrosis, submassive and recurrent submassive necrosis and postnecrotic scarring, but in my experiments these changes were not seen. Lane[8] observed extensive myocardial and hepatic injury in mice receiving the British yeast diet. Myocardial lesions were not observed in my experiments.

Summary

Seventeen male albino rats of the Lister strain were fed a diet containing 18% Japanese brewer's yeast as the sole source of dietary protein. One rat died of pneumonia. Eight rats (50%) died from massive hepatic necrosis after receiving the diet for 24 to 48 days (average: 33 days). The chief histological changes in the livers of rats which died from hepatic necrosis were massive coagulative necrosis with fatty infiltration and hemorrhage. It is concluded that Japanese brewer's yeast may be relatively capable of producing dietary hepatic necrosis, for it may be deficient in cystine, α-tocopherol and Factor 3.

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Cardiovascular Responses During Interruption of the Thoracic Inferior Vena Cava. An Experimental Study*

By

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(Received for publication, September 13, 1956)

Increasing number of operations for constrictive pericarditis, decortications of the lung, malignancies in the thoracic cavity and hepatic tumors have required considerable improvement in pathology, diagnosis, and operative technique. One of the imminent danger during these operations is injury to the great vessels. Recently attention on the superior vena cava syndrome has been brought to the medical men and corrective procedures have been proposed by few surgeons.[1] Management of the inferior vena cava in the thorax, however, still remains to be solved. In a recent communication,[2] we reported that transplantation of grafts in the thoracic inferior vena cava showed better results when the operation was completed within 30 minutes, otherwise the dogs did not survive the operation more than 10 hours. This finding prompted us to further investigate the cardiovascular response while the venous circulation above the diaphragm was completely interrupted.

Experimental

Method

21 adult mongrel dogs weighing 6–12 kg. were used for this experiment. Following intravenous anesthesia with 2.5% pentothal sodium (25–30 mg. per kg. body weight) via the femoral vein, endotracheal tube was inserted. The tube was connected to a closed circuit anesthesia machine and intermittent positive pressure with oxygen was obtained manually. Strict surgical asepsis was observed during the following operation. After the animals were placed on its left side, the 7th right intercostal space was opened widely with a rib retractor from the sternum to the posterior axillary line. While the right phrenic nerve

* Read in part at the 3rd General Meeting of the Japan Anesthesiology Society, Tokyo, October 20, 1956.

was separated from the inferior vena cava, contralateral chest cavity was always opened due to the anatomical relationship of the mediastinum to the vena cava. The inferior vena cava was obstructed between the right atrium and diaphragm either with two Satinsky's blood vessel clamp or a rubber tube fed with a plastic tube which was passed around the vessel and interrupted by pulling it upwards while pushing the rubber tube against the vena cava. Time of interruption lasted between 22–80 minutes. In several cases simultaneous interruption of the thoracic inferior vena cava with the descending thoracic aorta where the 7th intercostal artery branched off was performed for one hour. When the chest was closed, 200,000 units of penicillin was sprayed in the pleural cavity and the lung reinflated. Postoperatively, no antibiotics, blood and fluid was administered (Fig. 1).

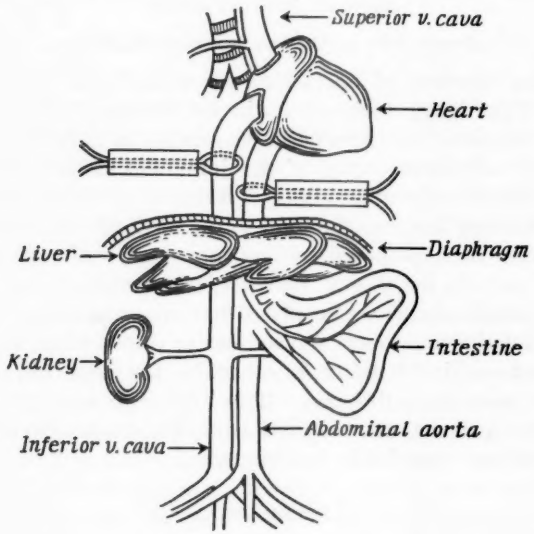

Fig. 1. Site of interruption of the thoracic vena cava and the descending thoracic aorta.

Hypothermia was obtained by immersing the animal in an ice water bath (4°C) below the neck after intravenous anesthesia with barbiturates together with insertion of the endotracheal tube. When the rectal temperature fell from 38°C to 35°C, usually within 20 minutes, the dog was removed from the tub and wiped off with dry cloths. Interruption of the inferior vena caval circulation was done between 29°C and 32°C, average 30.7°C. After the chest was closed, the dog was put into a hot water bath (43°C) for an hour till the rectal temperature rose to 38°C. Then it was removed from the tub and wiped thoroughly and kept in the animal house.

During this experiment, arterial blood pressure was measured with an U type mercury monometer with heparin 10 mg. in 5 cc. saline solution via the femoral or carotid artery, venous blood pressure with a water monometer with heparin via the femoral or external jugular vein. Observations were made on respiration and pulse rate. Post-operative autopsies were made and microscopic studies performed.

Results

21 experiments were carried out.

Temporary interruption of the thoracic inferior vena cava was performed under normal body temperature in 10 dogs (Table I). Two of 10 was permanently ligated and died 22 minutes and 30 minutes later, respectively. In all

TABLE I

Temporary Interruption of the Thoracic Inferior Vena Cava Under Normal Body Temperature (10 Dogs)

No. of experiment	Case	Time of interruption (min.)	Survival	Cause of death	Notes
1	No. 1(B)	50	36 hours	Shock	Graft transplanted
2	No. 2(B)	80	6 hours	Ditto	Ditto
3	No. 5(B)	25	20 days	Venography	Ditto
4	No. 9(B)	32	9 hours	Shock	Ditto
5	No. 10(B)	31	35 days	Venography	Ditto
6	No. 3(B)	42	0	Cardiac arrest	Ditto
7	No. 5(E)	30	11 hours	Shock	
8	No. 1(E)	30	0	Cardiac arrest	Permanent ligation
9	No. 2(E)	22	0	Ditto	Ditto
10	No. 10(E)	1 hour	74 hours	Unknown	Simultaneous ligation with descend. thoracic aorta

All grafts patent

cases cardiac arrest occurred. 7 cases were temporarily interrupted between 25 and 80 minutes, average 41 minutes. All but two died in shock stage within 11 hours after restoration of the venous circulation. Two died 20 days and 35 days later due to venography with 120% sodium iodide, respectively. Both cases (No. 5-B and No. 10-B) were interrupted for 25 minutes and 31 minutes, respectively. No. 10 (E) had one hour simultaneous ligation of the thoracic inferior vena cava with the descending thoracic aorta where the 7th right intercostal artery branched off. Following the release of both ties, it died 74 hours later with unknown reason.

Eleven dogs were subjected to temporary interruption of the thoracic inferior vena cava under hypothermia, average rectal temperature was 30.7 °C (Table II). Two of 11 was permanently ligated and died 33 minutes and 34 minutes later, respectively. Cardiac arrest occurred in both cases. 6 cases but

TABLE II

Temporary Interruption of the Thoracic Inferior Vena Cava Under Low Body Temperature (11 Dogs)

No. of experiment	Case	Time of interruption (min.)	Rectal temperature (°C)	Survival	Cause of death	Notes
1	No. 4(B)	20	31	31 days	Venography	Graft transplanted
2	No. 6(B)	36	32	1 hour	Haemorrhagic shock	Ditto
3	No. 7(B)	25	30	26 days	Venography	Ditto
4	No. 8(B)	25	32	18 hours	Pneumothorax	Ditto
5	No. 11(B)	28	30	4 days	Venography	Ditto
6	No. 4(E)	28	32	48 hours	Intestinal obstruction	
7	No. 3(E)	33	30	0	Cardiac arrest	Permanent ligation
8	No. 6(E)	34	31	0	Ditto	Ditto
9	No. 7(E)	1 hour	30	4 hours	Haemorrhagic shock	Simultaneous ligation with descend. thoracic aorta
10	No. 8(E)	Ditto	29	44 hours	Haemothorax	Ditto
11	No. 9(E)	Ditto	30	68 hours	Unknown	Ditto

All grafts patent

one were interrupted within 30 minutes, average 25.2 minutes. Five but one which died from pneumothorax survived over 24 hours. No. 6 (B) which was interrupted for 36 minutes died an hour later from haemorrhagic shock. Simultaneous ligation of the inferior vena cava with the descending thoracic aorta was carried out in three dogs. After one hour, the obstruction was completely released and normal passage was restored. Two of three (No. 7-E and No. 8-E) died due to excessive blood loss caused by technical error 4 hours and 44 hours after the operation, respectively. The remaining one No. 9 (E) died 68 hours later. The reason was not known even at autopsy.

The curves of blood pressure either under normal body temperature or low body temperature did not show remarkable difference. They will be described together.

Soon after the thoracic inferior vena cava was interrupted, vena cava distal to the obstruction markedly dilated together with engorgement of the diaphragmatic veins with blood. The movement of the diaphragm became limited due to the enlargement of liver. Femoral arterial blood pressure showed a rapid fall from 160 mmHg–150 mmHg to 50 mmHg between 2 and 5 minutes. Pulse pressure dropped from average 50 mmHg to average 10 mmHg., which continued for 6 minutes and gradually became smaller till cardiac arrest occurred. At first, tachycardia was observed for a few minutes. It was, however, followed with slow pulse and occasionally arryhthmia appeared 15 minutes later (Figs. 2 and 3). When obstruction was removed and free blood flow was established,

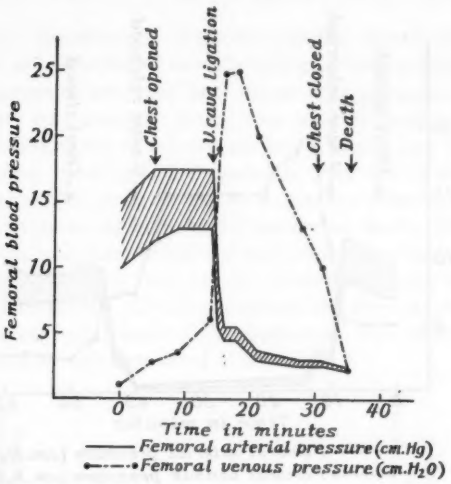

Fig. 2. Blood pressure changes during permanent ligation of the thoracic inferior vena cava under normal body temperature. Femoral arterial blood pressure dropped after the tie was placed and died 22 minutes later. This was in Case No. 2 (E).

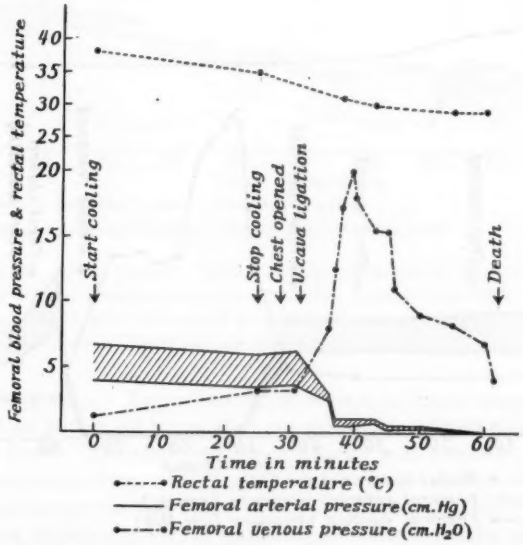

Fig. 3. Blood pressure changes during permanent ligation of the thoracic inferior vena cava under hypothermia at rectal temperature 30°C. Rapid fall of femoral arterial blood pressure occurred after the tie was placed and continued till death. This was in Case No. 3 (E).

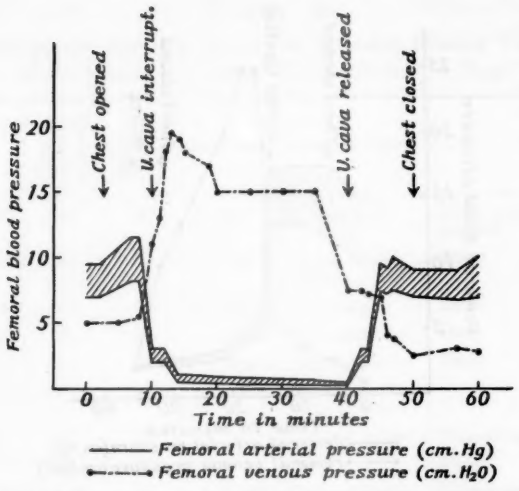

Fig. 4. Blood pressure changes during temporary interruption of the thoracic inferior vena cava under normothermia. Tie was released 30 minutes later and blood pressure returned to nearly normal level. This was in Case No. 5 (E).

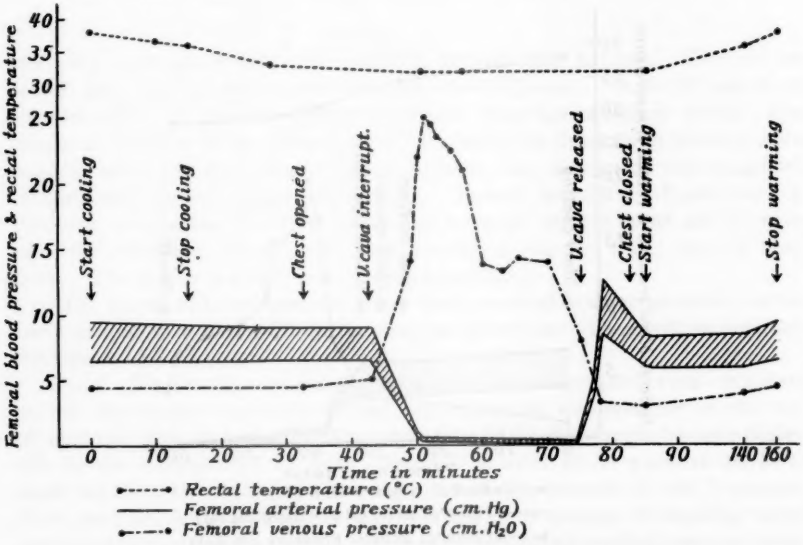

Fig. 5. Blood pressure changes during temporary interruption of the thoracic inferior vena cava under hypothermia at rectal temperature 32°C. Blood pressure curve resembled that which was observed under normothermia. This was in Case No. 4 (E).

stagnation rapidly disappeared. Femoral arterial blood pressure, however, remained as same as before for 40 seconds with gradual increase in systolic blood pressure. Pulse pressure increased and reached its maximum 2 minutes later. The blood pressure surpassed the preligation level 10 mmHg temporarily, and stablilized around 8 minutes to the normal level (Figs. 4 and 5). On the other hand, femoral venous blood pressure gradually rised with a rather sharp curve soon after the obstruction and reached its maximum between 2 and 4 minutes. The height was five times as high as the preligated level. Then it fell with a slow slope and some stabilized between 5 and 10 minutes at a 4/5 of the normal level while others gradually fell to the preligated level and died in case the block was not removed. On the contrary, the femoral venous blood pressure started to decline with release of the obstruction and returned to the normal level within 3 minutes and remained thereafter.

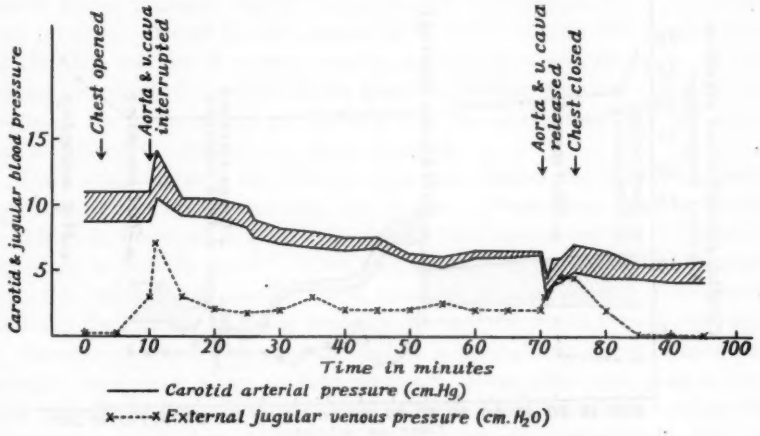

Fig. 6. Blood pressure changes during simultaneous ligation of the thoracic inferior vena cava with the descending thoracic aorta under normothermia. Slight fall of the carotid arterial blood pressure was only recognized during obstruction. This Case was found in No. 10 (E).

Macroscopic findings. Regardless of the normal or lower temperature group, those which died within 24 hours after the operation showed considerable congestion of the liver. Its surface was browny violet and granular. No bleeding was found on its cut surface. Pooling of blood in the portal bed was not evident. No petechiae or gangrene was found in the intestine and mesenterium. However, the serous membrane lost its glistening and slight edema was seen. The digestive tract did not contain blood. Spleen and kidney were both normal in its appearance.

Microscopic findings. Marked congestion of the liver was conspicuous. Though cells with vacuole were observed, necrosis was not evident. Congestion

of the spleen and kidney was slight. Only minor bleeding was found in the outer layer of the intestine.

When the thoracic inferior vena cava was ligated immediately following the descending thoracic aorta, the changes of blood pressures were quite different from that when the former was obstructed alone (Figs. 6 and 7). When both vessels were tied, carotid arterial blood pressure rose with a sharp curve accompanied with increasing pulse pressure for about 2 minutes and returned to the preligated level around 10 minutes. Transient arrythmia was usually observed 15 minutes later. Decrease of blood pressure became evident 40 minutes later and continuing decline was observed at the end of one hour. However, the fall was not over 35 mmHg.; and the initial blood pressure 110 mmHg was around 70 mmHg at the end of one hour. Following release of the thoracic

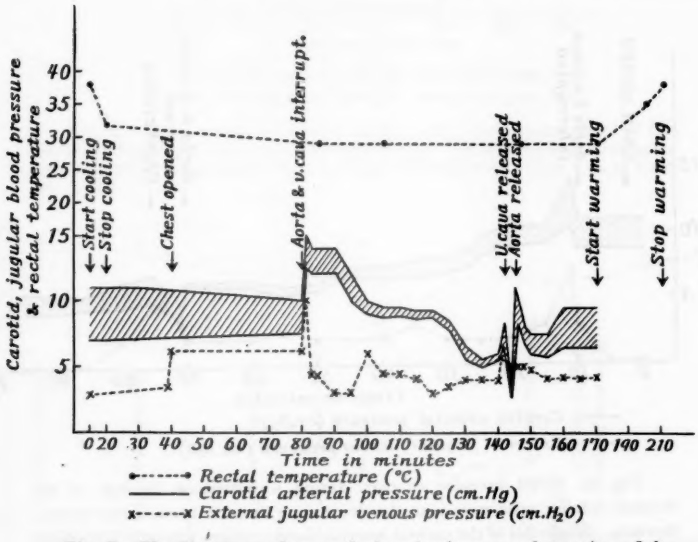

Fig. 7. Blood pressure changes during simultaneous obstruction of the thoracic inferior vena cava with the descending thoracic aorta under hypothermia at rectal temperature 29°C. No remarkable difference in blood pressure changes was noticed as that made under normothermia. This was recorded in Case No. 8 (E).

inferior vena cava and the descending thoracic aorta in the order, rapid fall of blood pressure immediately occurred at first and then rose higher than the preligated level temporarily. These changes were observed within two minutes and returned to the preoperative level 3–5 minutes later and gradually stabilized thereafter. Arrythmia disappeared about 10 minutes later and normal heart contraction was observed. When both vessels were tied, external jugular blood pressure rose 4 times as high as the preligated level and returned to the original

level 5 minutes later. Though minor fluctuations occurred with respiration and open chest, the entire course was relatively constant. Following transient rise with release of the ties, it returned to the preoperative level 2 minutes later. Though it was weak, spontaneous respiration was maintained during the obstruction period. The initial tachycardia existed only for a short time. Bradycardia was observed 50 minutes later, but returned to normal pulsation after restoring the circulation.

Macroscopic findings—No. 7 (E) and No. 8 (E) contained about 50 cc. and 60 cc. of blood in the pleural space, respectively. Otherwise all organs including that of No. 9 (E) and No. 10 (E) did not show congestion and other marked pathology, such as bleeding, necrosis etc.

Comment

When the thoracic inferior vena cava is obstructed suddenly, femoral arterial blood pressure rapidly declines and is accompanied with small pulse pressure. Heart hardly contracts 30 minutes after the obstruction and the dog will die if normal venous circulation is not restored. Considering the effect of obstructing the thoracic inferior vena cava, the return flow from the hepatic and portal bed, and the abdominal inferior vena cava including the kidney are both blocked.

Dale *et al.*[3] ligated the inferior vena cava below the kidney without any serious effect on the systemic circulation. Permanent ligation above the kidney is, however, known to be fatal to the animal within 10 hours.[4] According to Scheibe *et al.*[5], when this ligature is removed 150 minutes later, the animal will survive with changes of the central convoluted tubules of the kidney. On the contrary, in case the portal vein is obstructed, changes of the blood pressure follow a resembling curve when the thoracic vena cava alone is interrupted.[6] Soon after the obstruction, blood pressure of the portal vein distal to the ligature rises with a relatively sharp curve, while femoral arterial blood pressure immediately falls to shock level and always die if the tie remains over 30 minutes.[7] This is attributed to a large amount of blood loss into the portal circulation with decrease of general circulating volume,[8] destruction of blood contents, and release of toxin.[9] On the other hand, despite hepatic ciuculation has been obstructed on many occasions, total interruption is very difficult due to anatomical reasons. Child[10] said that hepatic vein could not be suddenly obstructed over 30–60 minutes with survival.

When we understand that the curves of blood pressure together with the time limit for survival which is about 30 minutes during interruption of the thoracic vena cava and portal vein are both following the same pattern, macroscopic picture is quite different from each other. That is, when the thoracic vena cava is tied permanently or over 30 minutes, liver is congested with small granules projected from its surface and ap-

pears dark browny violet, while the intestines and mesenterium are both slightly congested without necrosis or gangrene. Temperature did not show any effect on changes of blood pressure and general findings.

Elman *et al.*[8] and Peck *et al.*[6] demonstrated that simultaneous ligation of the portal vein with the superior mesenteric artery was effective in preventing fall of femoral arterial blood pressure. With this work in mind, we tied both descending thoracic artery and vena cava for 1 hour and found out that carotid arterial blood pressure didn't drop severely. This probably suggests that large volume of blood does not escape into the hepatic and portal bed. Studying the organs subjected to this procedure, a great amount of blood is stagnating in the liver rather than in the portal bed. Three of 4 lived over 24 hours. Two died from obvious blood loss which may be attributed to technical failure, while the remaining 2 died 68 hours and 74 hours, respectively from unknown reason.

The role of obstucting the aorta is briefly reviewd. Under normal body temperature, abdominal aorta can be safely ligated above the celiac axis for 20 minutes, above the superior mesenteric artery, 60 minutes.[11] Edwards *et al.*[12] explained that death due to obstruction became higher when the anoxic bed distal to the block became wider. This time limit can be prolonged by using hypothermia. The descending thoracic aort can be safely obstructed for 1 hour at rectal temperature 30°C without causing hindleg paralysis.[13] Bernhard *et al.*[14] demonstrated that the hepatic artery, the superior mesenteric artery and the portal vein were all ligated for 1 hour with all survival (27 dogs but 2) at rectal rempera-ture 24°C–27°C.

Causes of death after obstructing the thoracic or hepatic blood flow are attributed to hepatic necrosis due to anerobic bacterial growth,[15] product of V D M described by Shorr *et al.*,[16] haemorrahgic shock occurring in the liver,[17] or spinal cord damage. We could not, however, name these totally responsible for the late deaths which had temporary ties on the aorta and vein.

Though little effect was shown on the cardiovascular changes during normal—and low body temperature, the postoperative course was markedly different. In the normal body temperature group, the dogs recovered slowly from anesthesia after releasing the obstruction; and were apathic, lethargic and dyspeptic for 3 days, while in the lower body temperature group, they recovered from the anesthesia after the tie was removed and rectal temperature rose to 36°C. They ran and ate like other animals which underwent open thorax 24 hours later. The effect of hypothermia anesthesia is attributed to decrease of oxygen consumption of various organs.

Conclusions

21 experiments were performed to interrupt the thoracic inferior vena cava in dogs under normal- and lower body temperature.

1. Under normal body temperature, cardiac arrest occurrs when the thoracic inferior vena cava is obstructed over 30 minutes in dogs. Releasing the obstruction and restoring normal venous circulation, despite blood pressures will return to normal level, they die within 10 hours in shock. If the obstruction is removed before cardiac arrest occurrs, usually within 30 minutes, the animal will recover slowly from anesthesia, become lethargic, apathic and dyspeptic for 3 days. Then they are active.

2. Under hypothermia (average rectal temperature 30.7°C), cardiovascular responses are as same as that under normothermia. However, the dogs recover from anesthesia during warming process and act as same as those which underwent open chest surgery. They live like other normal dogs 24 hours later.

3. Shock may be attributed to large escaping of blood into the liver from the general circulation. Rapid fall of blood pressure during blocking the thoracic inferior vena cava can be effectively prevented by simultaneous ligation of the descending thoracic aorta for one hour with recovery.

Thanks are due to Prof. Shigetsugu Katsura for his suggestions. Dr. Takashi Kubo kindly assisted the operations. The expenses of this investigation were deprayed by a grant from the Scientific Research Fund of the Education Ministry, which is also gratefully acknowledged.

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Addendum: Since submission of this article, we became aware of the work by Owens, Liddle, Tune, & Zeavin, Surg. Forum (1953), 1954, 4, 63. Similar results were obtained.

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Studies on Discharge Intervals of a Single Motor Unit in the Human Extraocular Muscles

Part I. During Fixation of the Gaze

By

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(Received for publication, September 15, 1956)

The electrical activity of the extraocular muscles was observed with the rabbit as early as 1913 by Hoffman.[1] But no observation of it in the human subject had been made until recent years. Björk[2)-4)] was the first to work on and report about this problem. He made investigations during the years 1952–1954, on electromyography of the levator and the extrinsic eye muscles of man under normal and pathological conditions. Adler[5)] (1953), Magee[6),7)] (1954, 1956), Kamo-uchi[8)] (1955), Breinin and Moldaver[9),10)] (1955), Sakatani[11)-13)] (1955, 1956), Huber and Lehner[14)] (1956) also reported their own investigations on electromyography under normal or pathological conditions.

The author succeeded in 1953, in recording the action potentials of the human extraocular muscles under normal conditions, and reported it in the Japanese language.[15)-17)] The results conformed with Björk's observations in many points. However, the particular figures relating to the activity of a single motor unit have not yet been clarified by Björk and the others. As described by Björk, the extraocular muscles exhibit a constant electrical activity, not only in the primary position, but also as the antogonist during a slow movement of the eye. Hence the behavior of a single unit of the extraocula muscles forms a problem of much interest.

The present experiment was undertaken to obtain more precise informations about the behavior of a single unit of the extraocular muscles during fixation of the gaze.

Experimental

Materials and Methods

Observations were made on 15 adult subjects, aged 20 to 32 years, with normal vision and no affections of the eye muscles.

Fig. 1. Records of discharges from single units of the extraorcular muscles during fixation of the gaze : A, a part of the original record for Fig. 2 ; B, the same for Fig. 5 ; C, the same for Fig. 6. Time : 50 cyc./sec.

Fig. 2

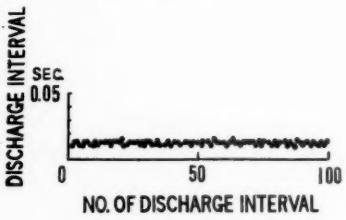


Fig. 3

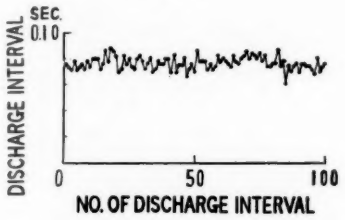


Fig. 2. Diagram of discharge intervals of a single unit of the internal rectus during fixation of the gaze.

Fig. 3. Diagram of discharge intervals of a single unit of the internal rectus during fixation of the gaze.

A monopolar needle electrode was used. It was a steel wire about 3.0 cm. long, 0.2 mm. in diameter, and sharpened with a file as fine as possible. It was insulated by varnishing with CASHEW (a paint made from lacquer), but the tip was left uncoated. The uncoated area of the tip was made approximately $10\,\mu \times 15\,\mu$, for the purpose of inducing the discharge of a single unit. To the butt of the needle was soldered a fine insulated copper wire.

The conjunctiva was anaesthetized with 0.5 per cent T-cain. The needle was inserted through the conjunctiva carefully into the muscle

Fig. 4

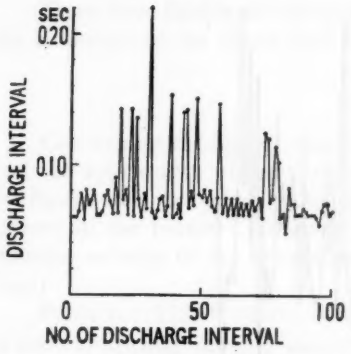
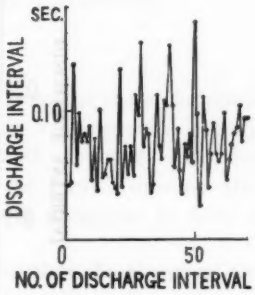
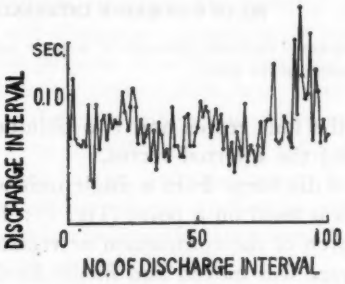


Fig. 4. Diagram of discharge intervals of a single unit of the internal rectus during fixation of the gaze.

Fig. 5





Figs. 5 and 6. Diagram of discharge intervals of a single unit of the external recuts during fixation of the gaze.

belly, but in case of examining the oblique muscles, it was inserted through the skin of the lid.

The experiments were carried out with the subjects in their supine position, and indifferent electrode was fixed on the tragus. A perimeter having a diameter of 60 cm. was settled before the eye in experiment, and the other eye was covered.

The action currents induced were amplified and recorded on a moving paper with an oscillograph of electromagnetic type, by observing the responses on a cathode-ray oscilloscope.

On the perimeter was fixed a white and round object of 1.0 cm. diameter, which could be moved along the arc of the perimeter.

Results

The action currents during fixation of the gaze in various directions

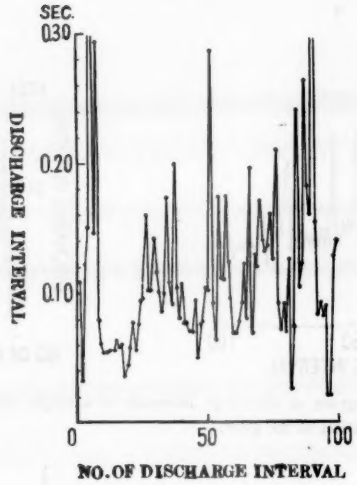


Fig. 7. Diagram of discharge intervals of a single unit of the internal rectus during fixation of the gaze.

were induced from the four rectus and two oblique muscles, but chiefly from the external and the internal rectus.

The frequency of discharge from a single unit was relatively constant, so long as the gaze was fixed on a point (Fig. 1); but it increased or decreased with the degree of the contraction or relaxation of the muscle in experiment, as the gaze was moved and finally fixed on another point.

In Figs. 2–7, are shown six diagrams of discharge intervals of a single unit.

Generally speaking, the variations of discharge intervals were very small, and the patterns of the diagrams were relatively uniform, while the average rate of discharge was over 30 per second (Fig. 2). But, when the rate of discharge fell below 30 per second, the variations of discharge intervals increased, and the patterns of the diagrams presented various types. (Figs. 3–6). Furthermore, when the rate of discharge fell below 10 per second, all the units presented strikingly increased variations and very irregular patterns of discharge intervals (Fig. 7).

In Fig. 3 is seen a type of diagram which shows variations rather small as compared with a large mean of discharge intervals. The type in Figs. 4 and 5 has large variations and, at the same time, a large mean of discharge intervals, but the two diagrams vary greatly in pattern.

The diagram in Fig. 6 shows slow undulation, whereas the gaze was fixed on a point in the experiment. The reason for the undulation is

unknown.

From these figures, it is supposed that there are some differences among the behaviors of the above four units (Figs. 3, 4, 5 and 6).

Discussion

Concerning the figure, amplitude and duration of the spike discharge, and the appearance of interference voltage, there are precise descriptions by Björk and the others, and my observations conform with them in almost all the points. However, the reason for Björk's opinion that the constant activity of the extraocular muscles will disappear in sleep is not clear.

Pulfrich (1952)[18] observed with the rabbit that the electrical activity of the extraocular muscles would not disappear before the deep stage of anaesthesia. The author, too, observed with the rabbit that it could be induced with ether even in the third stage of anaesthesia. From these observations, it is doubtful whether the electrical activity of the human extraocular muscles will disappear completely in natural sleep.

The variety of the patterns of interval diagrams is supposed to be due to the facts that each motor unit receives many nerve fibres of central and peripheral origins in different numbers and different kinds, and that their function therefore is very complicated, being related to many reflex arcs.

The slow undulation in the interval-diagram is also often seen in the other skeletal muscles, and is said to synchronize sometimes with respiration.[19] No experiment has been undertaken with the extrinsic eye muscles to investigate into its physiological origin.

Summary

The action potentials of normal extraocular muscles of a man were induced, and the discharge intervals of a single motor unit were observed during fixation of the gaze.

1. During fixation of the gaze on a point, the discharge frequency of a single unit is constant, but, if the gaze is moved to another point, it varies with the degree of contraction of the muscle in experiment.
2. When the average rate of discharge is over 30 per second, the variation of discharge interval is very small and the patterns of interval diagrams are uniform.
3. When the average rate of discharge falls below 30 per second, the variation of discharge interval increases and the patterns of diagrams give various types.
4. When the average rate of discharge is below about 10 per second, regularity of the discharge is scarcely found.

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Studies on Discharge Intervals of a Single Motor Unit in the Human Extraocular Muscles

Part II. During Horizontal Movement of the Eye

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Electromyography of the extraocular muscles has been attracted attentions of several workers recently (Part I). As already described by them, the most characteristic feature of the extraocular muscles is that they perform constant electrical activity, that is, in the primary position of the eye and even in the movement out of the muscle field.

An attempt has been made in order to clarify the mechanism of the constant activity of the extraocular muscles, by observing discharge intervals of a single unit. The foregoing report concerned the results of observation during fixation of the gaze (Part I). In this paper is reported the same problem probed during movement of the eye.

Experimental

Materials and Methods

Observations were made on 15 adult subjects with normal vision and no affection of the eye muscles.

A monopolar needle electrode was employed, the rest of the methods being the same as in the previous experiment (Part I).

Only the internal and the external rectus muscle were investigated during slow abducting and adducting movement of the eye. On a perimeter before the eye, a white and round object of 1.0 cm diameter was fixed, which however could be moved along the arc of the perimeter. In order to observe the action currents during movement, the object was moved by a handle at the rate of about 5°–10° per second, as smoothly as possible. The times of beginning and cessation of the movement were signalled by an assistant, and they were marked on the moving paper by another assistant.

Results

According to Cords,[1] slow movement of the eye without an object to fix its gaze upon is not carried out with smoothness, but in a stepping manner. However, movement with a moving object to fix its gaze is carried out with a considerable smoothness. The former type of movement is called impulsive movement, and the latter gliding movement.

Fig. 1

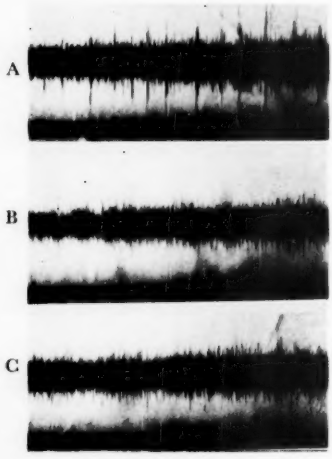

Fig. 2

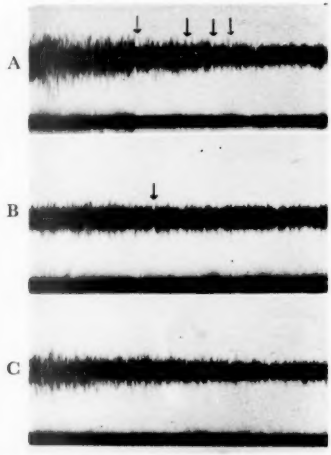

Fig. 1. Electrical responses of the right internal rectus (agonist) : A, during a slow horizontal movement of the eye from the right to the left, without object. Repeated bursts of discharge appeared ; B, during the same movement carried out by fixing the gaze on the object moving 5°~10° per sec ; C, during the movement carried out by turning the head to the right, fixing the gaze on the object straight ahead. Time : 50 cyc./sec.

Fig. 2. Electrical responses of the right internal rectus (antagonist) : A, during a slow horizontal movement of the eye from the left to the right, without object. Pauses of discharge were observed ; B, during the same movement carried out by fixing the gaze on the object moving 5°~10° per sec ; C, during the movement carried out by turning the head to the left fixing the gaze on the object straight ahead. Time : 50 cyc./sec.

Fig. 1 A and Fig. 2 A are interference voltages of the internal rectus induced in impulsive movements. Repeated bursts of discharges in the agonist (Fig. 1A), and repeated inhibitions in the antagonist (Fig. 2A) were observed concomitantly with repeated small impulsive movements. On the other hand, during so called gliding movements, either bursts or inhibitions of discharge were rarely observed (Figs. 1B and 1C; Figs. 2B and 2C). The phenomenon had been already described by Björk.

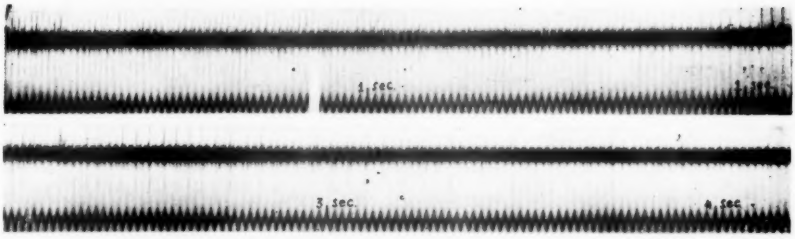

Fig. 3. Record of discharges from a single unit of the internal rectus (antagonist), during abducting movement of the eye : continued from above to below. It is the original record for Fig. 4. Note that the discharge intervals are lengthening gradually. Time : 50 cyc./sec.

Fig. 4

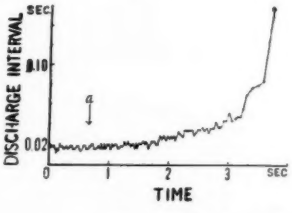

Fig. 5

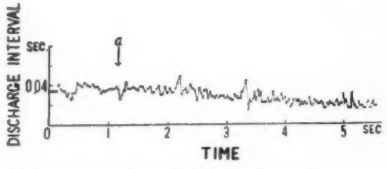

Fig. 4. Discharge intervals of a single unit of the right internal rectus (antagonist). The head was turned to the left, fixing the gaze on the object straight ahead. Onset of the movement, a.

Fig. 5. Discharge intervals of a single unit of the right internal rectus (agonist). The head was turned to the right, fixing the gaze on the object straight ahead. Onset of the movement, a.

Fig. 6

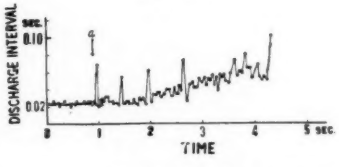

Fig. 7

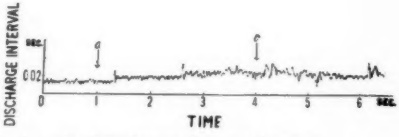

Fig. 6. Discharge intervals of a single unit of the internal rectus (antagonist), during the abducting movement of the eye from the primary position, without object. Onset of the movement, a.

Fig. 7. Discharge intervals o. a single unit of the internal rectus (antagonist), during the abducting movement of the eye from 5° to 15° abducted position, fixing the gaze on the moving object. Onset of the movement, a ; cessation of the movement, b.

The lines in Figs. 4–15 were obtained from the discharge of some units in agonists and antagonists during these two types of horizontal

Fig. 8

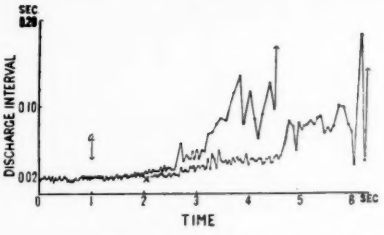

Fig. 9

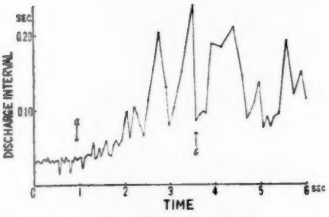

Fig. 8. Discharge intervals of two single units of the internal rectus (antagonist), during the abducting movement from the primary pisition, fixing the gaze on the moving object. Onset of the movement, a ; unknown, x.

Fig. 9. Discharge intervals of a single unit of the internal rectus (antagonist), during the abducting movement of the eye from 5° adducted to the primary position, without object. Onset of the movement, a ; cessation of the movement, b.

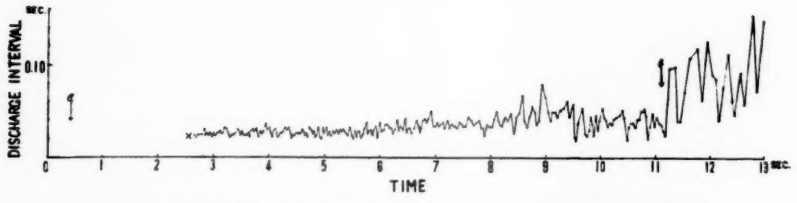

Fig. 10. Discharge intervals of a single unit of the internal rectus (antagonist), during the movement from the primary to the extremely abducted potion, fixing the gaze on the moving ofject. Onset of the movement, a ; cessation of the movement, b ; unknown, x.

movements. These lines indicate how the discharge interval changes with each unit. One of the original records is given (Fig. 3).

As a principle, the lines of agonists fall gradually, while those of antagonists rise.

Impulsive movement and gliding movement

Figs. 4, 5, 7, 8, 10 and 14 are obtained from gliding movements, while Figs. 6, 9 and 13 are from impulsive movements. Unexpectedly, when these two groups of the lines are compared with each other, no apparent difference is found between them.

The difference between the gliding and the impulsive movement does not always seem to occur in the behavior of each unit.

So the smoothness of the eye movement is considered owing not only to the smallness of variations of discharge intervals, but also to asynchronism of discharge of each unit and gradual recruitment or diminution of a unit

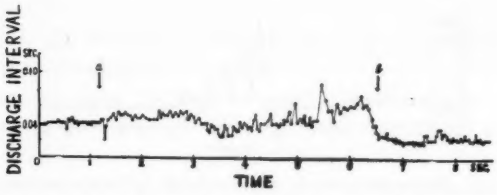

Fig. 11. Discharge intervals of a single unit of the external rectus (antagonist), during the movement from the primary to the extremely adducted position, fixing the gaze on the moving object. Onset of the movement, a ; cessation of the movement, b.

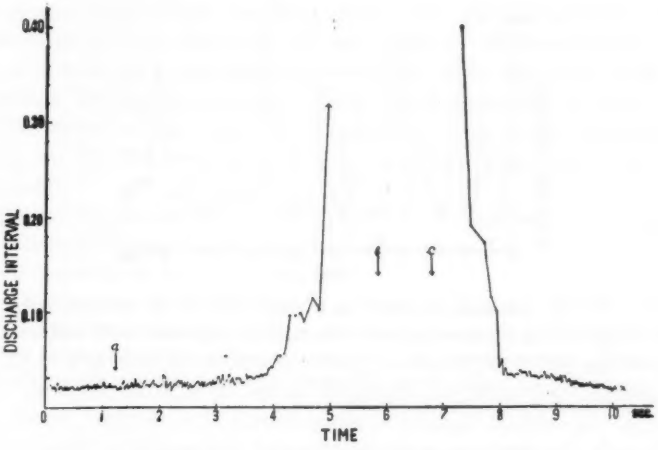

Fig. 12. Discharge intervals of a single unit of the internal rectus (antagonist—agonist), during the movement from the primary to 15° adbucted position, and returning again to the primary position, fixing the gaze on the moving object. Onset of the movement, a and c ; cessation of the movement, b ; unknown, ×.

in activity.

The range of action of each unit

During horizontal movement of the eye, related to the internal rectus, the motor units except a few units were observed to cease to discharge one after another, on the way to an extremely abducted position (Fig. 8).

The discharge of a single unit in the internal rectus, during an abducting movement of the eye, was observed to continue with very large intervals, when the movement was interrupted for a moment before the unit ceased to discharge (Fig. 9). But, when the abducting movement was continued further, the discharge of the unit ceased (Fig. 8). Further-

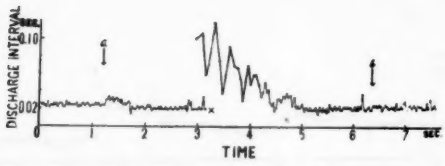

Fig. 13. Discharge intervals of two units of the internal rectus (agonist), during the movement from the primary position to 20° abducted position, without object. A new unit began to discharge two seconds after the onset of the movement. Onset of the movement, a ; cessation of the movement, b ; unknown, ×.

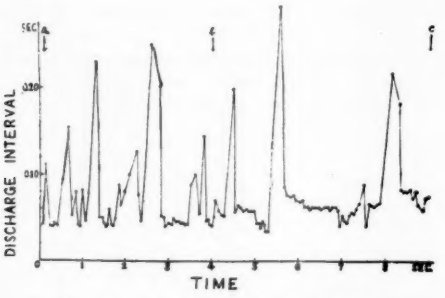

Fig. 14. Discharge intervals of a single unit of the external rectus (antagonist—agonist), during the movement from the primary to 25° adducted position, and returning again to the primary position, fixing the gaze on the moving object. Onset of the movement, a ; return of the movement, b ; cessation of the movement, c.

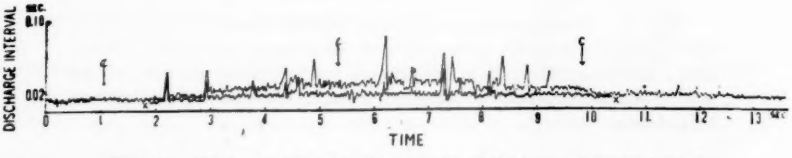

Fig. 15. Discharge intervals of two units of the internal rectus (antagonist), during the movement from the primary to 15° abducted position and returning again to the primary position, fixing the gaze to the moving object. Onset of the movement, a and c ; cessation of the movement, b ; unknown, ×.

more, when falling back, the adducting movement was carried on for a moment after the cease of discharge, the same unit began to act at approximately the same position of the eye where it ceased to discharge, with the corresponding intervals (Fig. 12).

From these observations, it is suggested that each unit has its own

range of action.

In this way, the increase of units in activity in agonist and the decrease of those in antagonist seem to take place.

Figs. 10 and 11 show the behaviors of a single unit in the antagonist which continued to discharge, in the extremest movement of the eye out of the muscle field.

The unit shown in Fig. 10 decreased its rate of discharge while the agonistic muscle was contracting as strongly as possible. The unit in Fig. 11, on the contrary, increased the rate of discharge. The type in Fig. 10 was observed rather frequently, while the type in Fig. 11 was rare.

Common figures of a single unit's activity

It is noteworthy that the ascendence and the descendence of the lines are often not parallel to the eye movements. As seen in Figs. 10, 12 and 13, the lines are remaining horizontal for some period of time, notwithstanding the eye movement. From these patterns, it seems likely that all or many of the units are discharging with rather constant and high frequencies, independently of the eye movement, within the range of their own.

With the beginning and the cessation of the movement, unexpectedly few characteristic figures were observed (Figs. 6 and 9).

Some characteristic patterns of line

1) Some units in the antagonist, as seen in Fig. 14, showed repeated pauses of discharge instead of decrease in the rate of discharge. In the latter half of Fig. 14, the muscle is acting as an agonist, but the base line does not descend, though pauses of discharge are seen less frequent.

2) Some units in antagonists show a stepped ascendence of a line, each step being accompanied by a larger discharge interval (Fig. 7).

Relations between the two units

Lines of two motor units obtained simultaneously from a single muscle are shown in Figs. 8, 13 and 15. These pairs were all induced with a single needle electrode.

The pairs in Figs. 8 and 13 look like acting independently of each other, and carrying on the division of work. In Fig. 15, however, the large discharge intervals of both units are sometimes synchronized and sometimes not. Consequently, the two units are sometimes cooperative, but sometimes independent.

Discussion

The author hesitates to agree with Breinin and Moldaver,[2] who assert that the firing of inhibited muscles falls off to zero in the extremest movement of the eye out of the muscle field. The author's investigations proved that electrical activity of the antagonistic muscles never falls off

to zero in the like movement. In this point, the author is of Björk's[3] opinion.

It is interesting to notice that a few units in the antagonist were observed to increase in frequency of discharge in the extremest movement of the eye out of the muscle field (Fig. 11). This may be explained as a case of reflex fixation or, perhaps, of stretch reflex.[4]

That some motor units continue, independently of the eye movement, to discharge with very high frequencies, must be one of the most important natures of a unit in the extraocular muscles. Björk had also described this phenomenon.

Regarding such patterns of lines, as, relatively horizontal course independent of the eye movement, stepped ascendence, increase of discharge frequency in some units in the antagonist during the extremest movement of the eye, any unit in the internal or the external rectus does not seem to be always faithfull to the law of reciprocal innervation.

Creed and the others[5] had noticed that contraction of a muscle exhibits a compromise between the two opposing effects, an " algebraical summation " of central excitation and central inhibition. From this point of view, it is worthy of notice that the behavior of each unit is not only different from those of others, but seems to have some opposing effects in itself.

Summary

The action potentials of internal and external rectus muscles of a man were induced during a slow horizontal movement of the eye, and the behavior of a single motor unit were investigated by observing its discharge intervals.

1. During a horizontal movement of the eye, discharge intervals of a single unit in the agonist as a rule, decreased, and those in the antagonist increased, but the behavior of each unit varied so much.

2. A striking fact is that some units were often observed discharging with rather constant and high frequencies, regardless of the movement of the eye.

3. Each unit of the internal and of the external rectus muscle had its own range of action fixed to the movement of the eye.

4. The difference between the impulsive and the gliding movement did not always appear in the behavior of each single unit.

5) In the extremest movement of the eye out of the muscle field, the activity of the antagonistic muscle never fell off to zero.

6. Moreover, a few units in the antagonistic muscle were sometimes observed to increase its rate of discharge in the same movement.

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